Design, Synthesis, Characterization and Biological evaluation of Benzothiazole fused Guanidinopropionic ester derivatives

Dissertation Submitted to

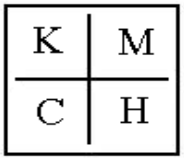
The Tamil Nadu Dr. M.G.R Medical University, Chennai

In partial fulfillment for the requirement of the Degree of

MASTER OF PHARMACY

(Pharmaceutical Chemistry)

April - 2012



DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

KMCH COLLEGE OF PHARMACY

KOVAI ESTATE, KALAPATTI ROAD,

COIMBATORE 641-048

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Submitted by

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Under the guidance of

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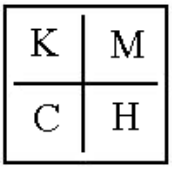

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY,

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CERTIFICATE

This is to certify that the dissertation work on ***" Design,* Synthesis*, Characterization and Biological evaluation of Benzothiazole fused Guanidinopropionic ester derivatives"*** submitted by **Ms. S. Saranya** is a bonafide work carried out by the candidate under the guidance of **Prof. K. Suresh Kumar, M. Pharm, (Ph.D.,)** to ***The Tamil Nadu Dr. M.G.R. Medical University, Chennai,*** in partial fulfillment for the degree of **Master of Pharmacy** in Pharmaceutical Chemistry at the Department of Pharmaceutical Chemistry, KMCH College of Pharmacy, Coimbatore, during the academic year 2011-2012.

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"She carries my best wishes"

Prof. K. Suresh Kumar, M. Pharm, (Ph.D).

DECLARATION

I do hereby declare that the dissertation work entitled ***"Design, Synthesis, Characterization and Biological evaluation of Benzothiazole fused Guanidinopropionic ester derivatives"*** submitted to ***The Tamil Nadu Dr. M.G.R. Medical University, Chennai***, in partial fulfillment for the Degree of **Master of Pharmacy** in Pharmaceutical Chemistry at the Department of Pharmaceutical Chemistry was done by me under the guidance of **Prof. K. Suresh Kumar, M. Pharm, (Ph.D).,** at the Department of Pharmaceutical Chemistry, KMCH College of Pharmacy, Coimbatore, during the academic year 2011-2012.

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EVALUATION CERTIFICATE

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Examination Centre: KMCH College of Pharmacy, Coimbatore.

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Above all I dedicate myself before the unfailing presence of ***GOD*** and constant love and encouragement given to me by my beloved **Parents, Sisters & Anush** who deserves the credit of success in whatever work I did.

S. SARANYA

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INTRODUCTION

DIABETES MELITUS

Diabetes is a condition in which the body does not produce or respond to insulin, a hormone that regulates the level of sugar in the blood. Diabetes mellitus arises when insufficient insulin is produced or when the available insulin does not function correctly. Without insulin, the amount of glucose in the blood stream is abnormally high, causing unquenchable thirst and frequent urination. The body's inability to store or use glucose causes hunger and weight loss.

HISTORY[1]

Physicians have observed the effects of diabetes for thousands of years. For much of this time, little was known about this fatal disease that caused wasting away of the body, extreme thirst and frequent urination. It wasn't until 1922 that the first patient was successfully treated with insulin.

One of the effects of diabetes is the presence of glucose in the urine (glucosuria). Ancient Hindu writings, many thousands of years old, document how black ants and flies were attracted to the urine of diabetics. The Indian physician Sushruta in 400 B.C. described the sweet taste of urine from affected individuals and for many centuries to come, the sweet taste of urine was key to diagnosis.

Around 250 B.C., the name “diabetes” was first used. It is a Greek word that means “to Syphon”, reflecting how diabetes seemed to rapidly drain fluid from the affected individual. The Greek physician Aretaeus noted that as affected individuals wasted away, they passed increasing amounts of urine as if there was “liquefaction of flesh and bones into urine”. The complete term “diabetes mellitus” was coined in 1674 by Thomas Willis, personal physician to King Charles II. Mellitus is Latin for honey, which is how Willis described the urine of diabetics (“as if imbued with honey and sugar”).

Up until the mid-1800s, the treatments offered for diabetes varied tremendously. Various “fad” diets were prescribed and the use of opium was suggested, as were bleeding and other therapies. The most successful treatments were starvation diets in which calorie intake was severally restricted. Naturally, this was intolerable for the patient and at best extended life expectancy for a few years.

A breakthrough in the puzzle of diabetes came in 1889. German physicians Joseph von Mering and Oskar Minkowski surgically removed the pancreas from dogs. The dogs immediately developed diabetes. Now that a link was established between the pancreas gland and diabetes, research focused on isolating the pancreatic extract that could treat diabetes.

To concentrate what we now know as insulin, Banting tied the pancreatic ducts of dogs. The pancreatic cells that released digestive enzymes (and could also destroy insulin) degenerated, but the cells that secreted insulin were spared. Over several weeks the pancreas degenerated into a residue from which insulin could be extracted. In July 1921, a dog that had its pancreas surgically removed was injected with an extract collected from a duct-tied dog. In the two hours that followed the injection, the blood sugar level of the dog fell and its condition improved. Another de-pancreatized (diabetic-like) dog was kept alive for eight days by regular injections until supplies of the extract, at that time called "isletin", were exhausted.

Further experiments on dogs showed that extracts from the pancreas caused a drop in blood sugar, caused glucose in the urine to disappear, and produced a marked improvement in clinical condition. So long as the extract was being given, the dogs were kept alive. The supply of the extract was improved: the pancreases of different animals were used until that of the cow was settled upon. This extract kept a de-pancreatized dog alive for 70 days. Dr. J. Collip, a biochemist, was drafted to continue improving the purity of the pancreas extract, and later, best carried on this work.

A young boy, Leonard Thompson, was the first patient to receive insulin treatment. On January 11, 1922, aged 14 and weighing only 64 pounds, he was extremely ill. The first injections of insulin only produced a slight lowering of blood sugar level. The extract still was not pure enough, and abscesses developed at the injection site. Collip continued to refine the extract. Several weeks later, Leonard was treated again and showed a remarkable recovery. His blood sugar levels fell; he gained weight and lived for another 13 years. He died from pneumonia at the age of 27.

During the spring of 1922, best increased the production of insulin to enable the treatment of diabetic patients coming to the Toronto clinic. Over the next 60 years, insulin was further refined and purified, and long-acting and intermediate types were developed to provide more flexibility. A revolution came with the production of recombinant human DNA insulin in 1978. Instead of collecting insulin from animals, new human insulin could be synthesized.

In 1923, Banting and Macloed were awarded the Nobel Prize for the discovery of insulin. Banting split his prize with Best and Macloed split his prize with Collip. In his Nobel Lecture, Banting concluded the following about their discovery.

“Insulin is not a cure for diabetes; it is a treatment. It enables the diabetic to burn sufficient carbohydrates, so that proteins and fats may be added to the diet in sufficient quantities to provide energy for the economic burdens of life.”

STATISTICAL DATA

The most common forms of diabetes are type 1 diabetes (5%) [2], which is an autoimmune disorder and type 2 diabetes (95%) [3], which is associated with obesity. Over 18 million Americans have diabetes; of these, about 5 million do not know they have the disease.

When people think of epidemics, they often think of infectious diseases such as SARS, HIV or the flu. However, the prevalence of type 2 diabetes is now at epidemic proportions. In the United States, diabetes accounts for over 130 billion dollars of health care costs and is the fifth leading cause of death. The number of new cases being diagnosed continues to rise. It has been estimated that of the children born in the year

2000, 1 of 3 will suffer from diabetes at some point in their lifetime. Diabetes is predicted to become one of the most common diseases in the world within a couple of decades, affecting at least half a billion people.

In the past, type 2 was rarely seen in the young, hence its original name of "adult-onset diabetes". But now type 2 diabetes is increasingly being diagnosed in young adults and even in children. In Japan, more children suffer from type 2 than type 1 ("juvenile onset") diabetes. This young generation of diabetics will have many decades in which to develop the complications of diabetes. In 1990, 4.9% of the American populations were diagnosed with diabetes. This increased to 7.9% by the year 2001[4].

Type 2 diabetes comprises 90% of people with diabetes around the world and is one of the major public health challenges of the 21st century. The number of cases worldwide in 2000 is estimated to be about 171 million and is projected to rise to 366 million in 2030. The World Health Organization (WHO) projects that without urgent action, diabetes-related deaths will increase by more than 50% in the next 10 years. Especially in upper-middle income countries, diabetes deaths are projected to increase by over 80% between 2006 and 2015.

TYPES

The three main types of diabetes are:

Type 1 diabetes

Type 2 diabetes

Gestational diabetes.

Type 1 diabetes

Type 1 diabetes (once known as insulin-dependent diabetes mellitus or juvenile diabetes) is considered an autoimmune disease. An autoimmune disease results when the body's system for fighting infection (the immune system) turns against a part of the body. In diabetes, the immune system attacks the insulin-producing beta cells in the pancreas and destroys them. The pancreas then produces little or no insulin.

Type 1 diabetes develops most often in children and young adults, but the disorder can appear at any age. Symptoms of type 1 diabetes usually develop over a short period, although beta cell destruction can begin years earlier.

Type 2 diabetes

The most common form of diabetes is type 2 diabetes (once known as noninsulin-dependent diabetes mellitus or NIDDM). About 90 to 95 percent of people with diabetes have type 2 diabetes. This form of diabetes usually develops in adults over the age of 40 and is most common among adults over age 55. About 80 percent of people with type 2 diabetes are overweight.

In type 2 diabetes, the pancreas usually produces insulin, but for some reason, the body cannot use the insulin effectively. The end result is the same as for type 1 diabetes--an unhealthy buildup of glucose in the blood and an inability of the body to make efficient use of its main source of fuel.

Gestational Diabetes

Gestational diabetes develops or is discovered during pregnancy. This type usually disappears when the pregnancy is over, but women who have had gestational diabetes have a greater risk of developing type 2 diabetes later in their lives.

SYMPYTOMS

Symptoms of Type I diabetes may include:

- increased thirst and urination
- constant hunger
- weight loss
- blurred vision
- Extreme tiredness.

Symptoms of Type 2 diabetes may include:

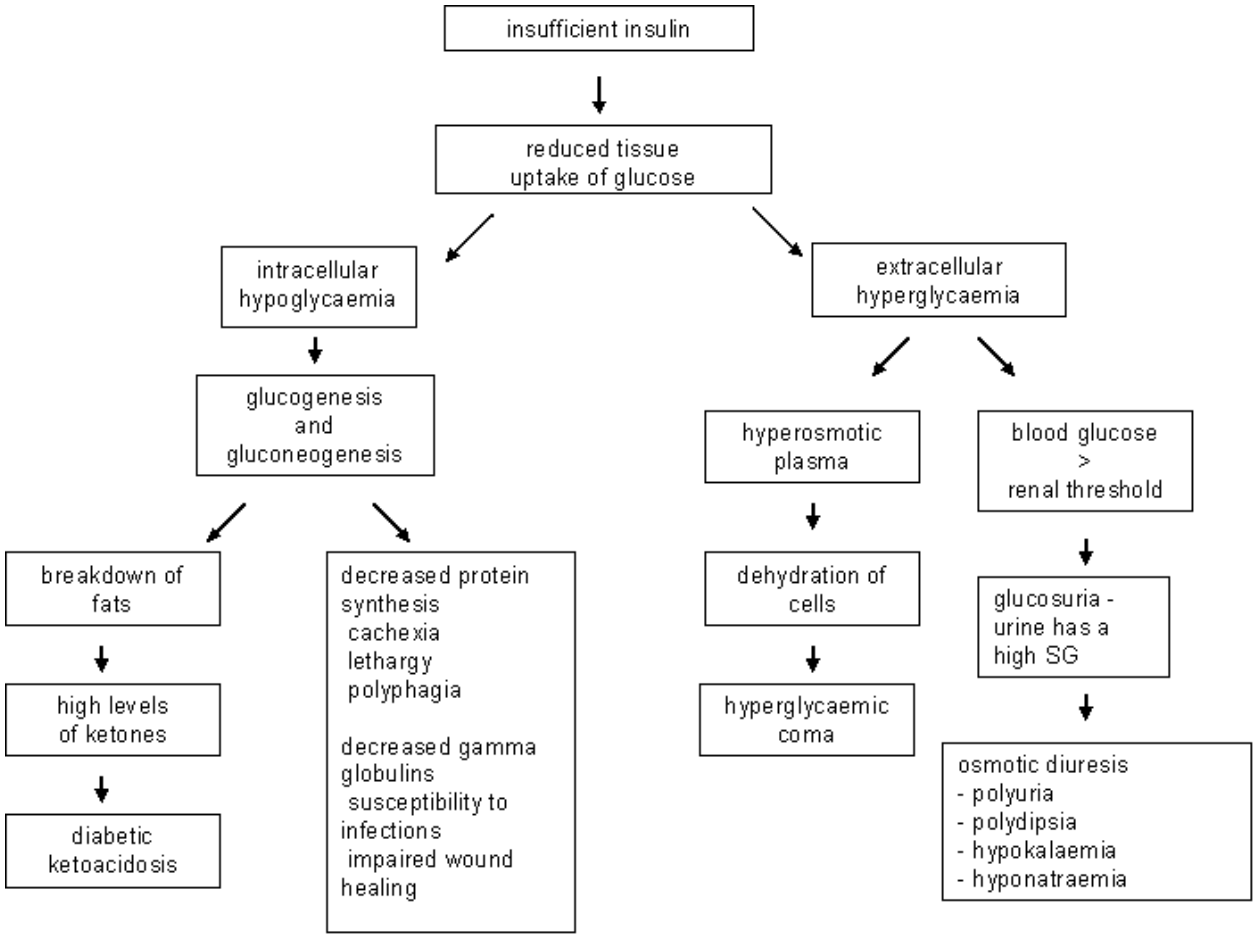
- feeling tired or ill
- frequent urination (especially at night)
- unusual thirst
- weight loss
- blurred vision
- frequent infections
- Slow healing of sores.
- having dry, itchy skin, tingling in the feet

CAUSES

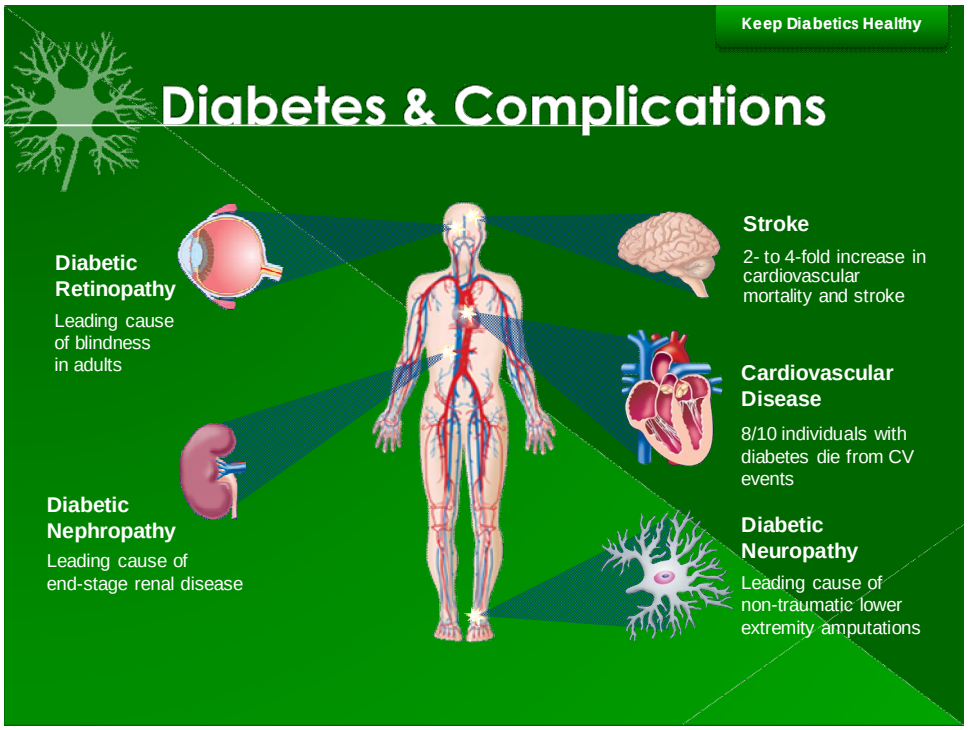
In type 1 diabetes, the insulin-producing beta cells are destroyed by an autoimmune process, whereby the body's immune system - its defense mechanism against disease for some reason recognizes the cells as being 'foreign' rather than 'self' and therefore attacks them.

Type 2 diabetes is thought to be due both to defects in the islet beta cells, so that less glucose is produced and to an impairment of insulin's ability to stimulate the uptake of glucose in muscles and other tissues. There is a genetic influence, as type 2 diabetes tends to run in families even more strongly than type 1 diabetes and several genes are likely to be involved. But increasing age, obesity and a sedentary lifestyle also increase the risk of type 2 diabetes.

PATHOPHYSIOLOGY



COMPLICATIONS



TREATMENT

The goal of diabetes management is to keep blood glucose levels as close to normal as safely possible. Since diabetes may greatly increase risk for heart disease and peripheral artery disease, measures to control blood pressure and cholesterol levels are an essential part of diabetes treatment as well.

Dietary Management and Physical Activity

Modifying eating habits and increasing physical activity are typically the first steps toward reducing blood sugar levels. At UCSF Medical Center, all patients work with their doctor and certified dietician to develop a dietary plan. Our Teaching Center conducts workshops that provide patients with information on food nutrient content, healthy cooking and exercise.

Insulin Therapy

People with type 1 diabetes require multiple insulin injections each day to maintain safe insulin levels. Insulin is often required to treat type2 diabetes too. Using an insulin pump is an alternative to injections. The pump is about the size of a pager and is usually worn on your belt. Insulin is delivered through a small tube (catheter) that is placed under the skin (usually in the abdomen).

There are four major types of insulin:

- Rapid-acting
- Short-acting
- Intermediate-acting
- Long-acting

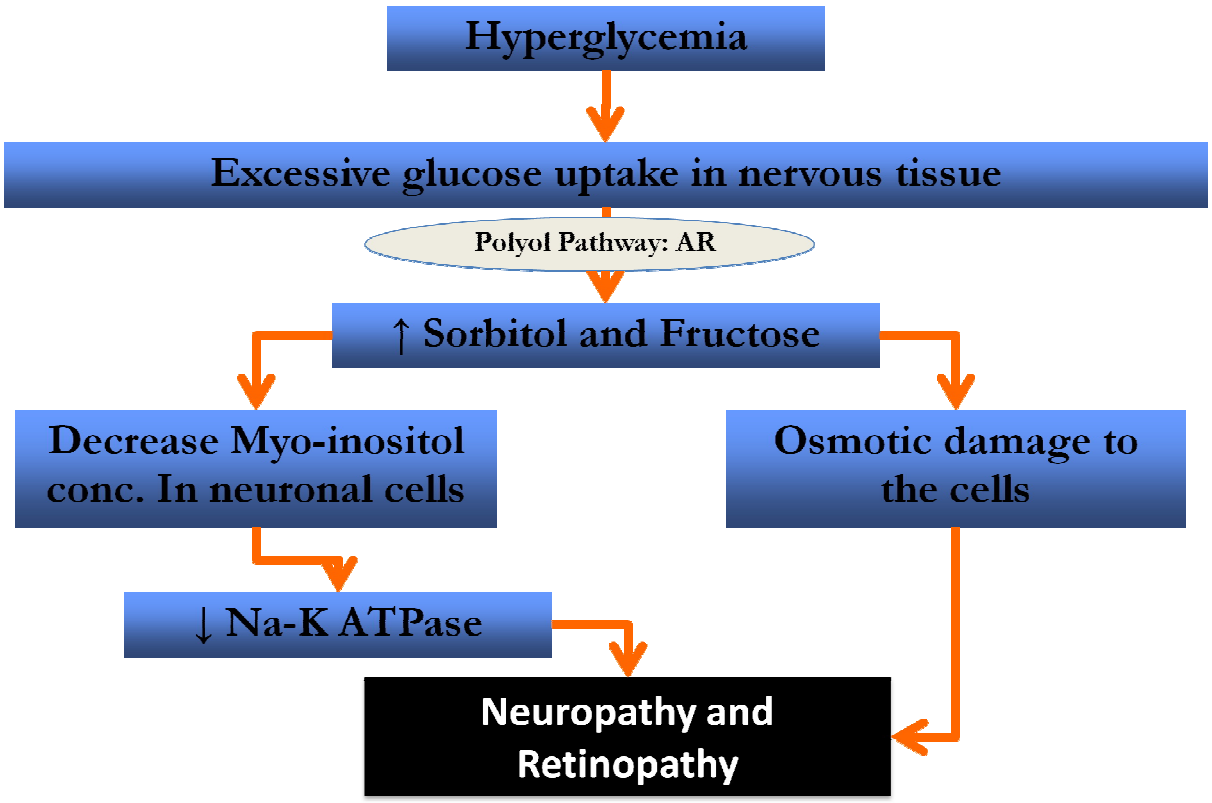
Your doctor will determine your dose and how often you need to take insulin. There is no standard insulin dose as it depends on factors such as your body weight, when you eat, how often you exercise and how much insulin your body produces.

Oral Medications

Sometimes blood sugar levels remain high in people with type 2 diabetes even though they eat in a healthy manner and exercise. When this happens, medications taken in pill form may be prescribed. The medications work in several different ways. These include improve the effectiveness of the body's natural insulin, reduce blood sugar production, increase insulin production and inhibit blood sugar absorption. Oral diabetes medications are sometimes taken in combination with insulin.

Aldose Reductase Inhibitor[5]

Aldose reductase (AR) inhibitor is a class of drugs being studied as a way to prevent eye and nerve damage in people with diabetes.



During Hyperglycemia, glucose entering the polyol pathway is reduced to sorbitol by Aldose reductase (AR) and NADPH. Sorbitol is subsequently oxidized to fructose by sorbitol dehydrogenase and NAD+. This increased flux through the polyol pathway results in a change in redox potential for these cofactors. The increased ratio of cytosolic NADH to NAD+ is called reductive stress, which has been linked to depleted intracellular levels of glutathione, increased nonenzymatic glycation and activation of protein kinase C. In addition to reductive stress, accumulation of sorbitol in certain cells results in osmotic stress, which can lead to cell swelling and eventually cell death. Inhibitors of aldose reductase can prevent these metabolic and biochemical changes, which have been linked to the pathogenesis of diabetic complications.

ANTIOXIDANT

The adverse effects of oxidative stress on human health have become a serious issue. The World Health Organization (WHO) has estimated that 80% of the earth's inhabitants rely on traditional medicine for their primary health care needs and most of this therapy involves the use of plant extracts and their active components. Under stress, our bodies produce more reactive oxygen species (ROS) (e.g., superoxide anion radicals, hydroxyl radicals and hydrogen peroxide) than enzymatic antioxidants (e.g., superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase) and non-enzymatic antioxidants (e.g., ascorbic acid (vitamin C), tocopherol (vitamin E), glutathione, carotenoids and flavonoids). This imbalance leads to cell damage[6] and health problems[7]. A lack of antioxidants, which can quench the reactive free radicals, facilitates the development of degenerative diseases[8], including cardiovascular diseases, cancers[9], neurodegenerative diseases, Alzheimer's disease and inflammatory diseases[10]. One solution to this problem is to supplement the diet with antioxidant compounds that are contained in natural plant sources[11]. These natural plant antioxidants can therefore serve as a type of preventive medicine. Recent reports indicate that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human disease[12]. However, synthetic antioxidants such as Butylated Hydroxy Toluene (BHT) and Butylated Hydroxy Anisole (BHA) have been widely used as antioxidants in the food industry and may be responsible for liver damage and carcinogenesis[13].

Antioxidants including phenolic compounds (e.g., flavonoids, phenolic acids and tannins) have diverse biological effects such as anti-inflammatory, anti-carcinogenic and anti-atherosclerotic effects as a result of their antioxidant activity[14].

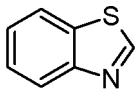
An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions. They do this by being

oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols[15].

Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells.
As oxidative stress might be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. However, it is unknown whether oxidative stress is the cause or the consequence of disease.

Reactive oxygen species (ROS) capable of causing damage to DNA has been associated with carcinogenesis, coronary heart disease and many other health problems related to advancing age[16]. In low concentrations, synthetic antioxidants are also in use for many industrial processes e.g. inhibition of radical formation for preventing premature polymerization during processing, storage and transportation of unsaturated monomers. They exert their effects by scavenging or preventing the generation of ROS[17] which can protect the formation of free radicals and retard the progress of many chronic diseases[18] including cancer, neurodegenerative, inflammation and cardiovascular diseases[19].

CHEMISTRY OF BENZOTHIAZOLE NUCLEUS



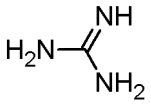
Being a heterocyclic compound, Benzothiazole finds use in research as a starting material for the synthesis of larger, usually bioactive structures. Its aromaticity makes it relatively stable, although as a heterocyclic, it has reactive sites, which allow for functionalization. Benzothiazole is a colorless, slightly viscous liquid with a boiling point of 227-228^{0}C. The density of Benzothiazole is 1.644 gm/ml and molecular mass is 139.19.

A huge number of therapeutic agents are Benzothiazole derivatives. During recent years there have been some interesting developments in the biological activities of Benzothiazole derivatives. These compounds have special significance in the field of medicinal chemistry due to their remarkable pharmacological potentialities.

The small and simple Benzothiazole nucleus is present in compounds involved in research aimed at evaluating new products that possess interesting biological activities like antidiabetic[20], antitumour[21], antimicrobial[22], antitubercular[23], antimalarial[24], anticonvulsant[25], anthelmintic[26], analgesic[27] and anti-inflammatory activity[28]. Heterocyclic containing the Thiazole moiety are present in many natural products such as Bleomycin, Epothilone, Lyngbyabellin A & Dolastatin.

Substituted 2-arylbenzothiazoles have emerged in recent years as an important pharmacophore in non-invasive diagnosis of Alzheimer's disease. Recently, benzothiazole derivatives have been evaluated as potential amyloidal-binding diagnostic agents in neurodegenerative disease and as selective fatty acid amide hydrolase inhibitors[29].

CHEMISTRY OF GUANIDINE NUCLEUS



The guanidine group defines properties of many biologically active compounds. Synthetic guanidines have found applications in the design of molecular recognition devices, sensors and catalysts as well as disinfectants and antiseptics for clinical use, and in the manufacture and preservation of industrial products[30]. Guanidines are among the strongest organic bases, which is associated with the charge delocalization via six *p*-electrons across the CN_3 unit[31].

Guanidine group stem from its unique basicity and it's planar, fork like structure. The guanidine group is capable of forming both electrostatic and directed hydrogen bond interactions with polar molecules and anions. Arginine, the only natural amino acid bearing the guanidinium functionality, is the most basic of all natural amino acids and has the highest proton affinity among amino acids. Since guanidine remains protonated over a wide pH range, including physiological pH (the pKa of unsubstituted guanidine is 13.5[32] and also possesses a geometry enabling it to align well with carboxylates, phosphates, sulfates, nitrates and other anionic groups in water as well as polar compounds in organic solvents.

Guanidine-containing molecules display a wide range of biologically important roles, a number of synthetic pharmaceuticals incorporate the guanidine framework as exemplified by the influenza inhibitor zanamivir[33]. In addition, in aqueous media the protonated guanidine is highly stable and is at the hear of the formation of selective non-covalent associations with anionic complementary groups[34]. Considering the growing importance and applications of guanidine derivatives in the field of medicinal and supramolecular chemistry, a continuous synthetic interest has been shown for the conversion of amines to the corresponding guanidines[35].

In view of above we are here attempts to fuse Benzothiazole with Guanidinopropionic ester for screening of anti-diabetic, antioxidant and antimicrobial activities.

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Chapter 2

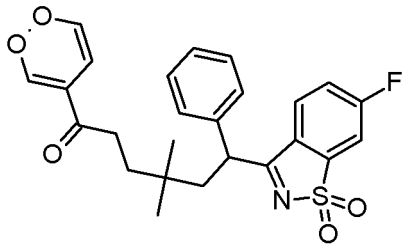
Literature Review

LITERATURE REVIEW

2. 1. BENZOTHIAZOLE DERIVATIVES

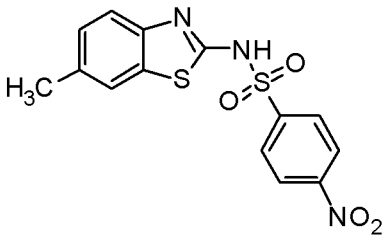
2. 1. 1. Anti-diabetic

Curt D[1] *et al* (2010), series of novel substituted N-{3-[(1, 1-dioxido-1, 2- benzothiazol-3-yl) (phenyl) amino]propyl} benzamide analogs (1) were synthesized and evaluated for anti-diabetic activity against Kv1.3 ion channel.

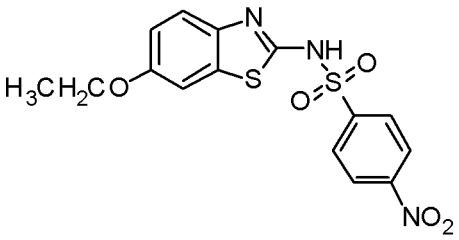


(1)

Gabriel Navarrete-Vazquez[2] *et al* (2009), synthesized 2-arylsulfonylaminobenzothiazole derivatives and *in vitro* inhibitory activities of the synthesized compounds were evaluated against protein tyrosine phosphatase 1B (PTP-1B). Among them, Compounds (2) and (3) showed good inhibitory activity and *in vivo* anti- hyperglycemic activity in a type 2 diabetes mellitus rat model.

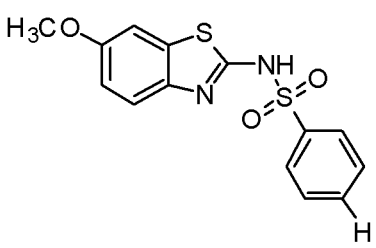


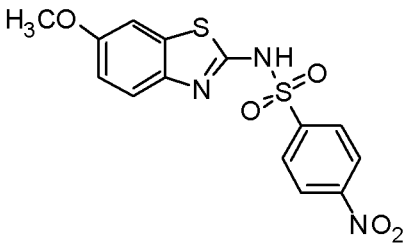
(2)



(3)

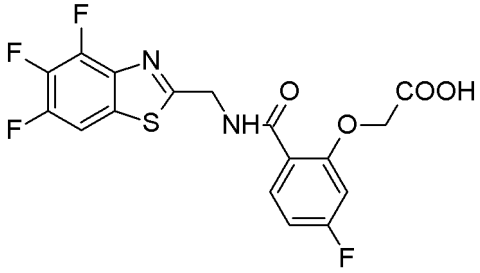
Hermenegilda Moreno-Dıaz[3] *et al* (2008)**,** synthesized -N-(6-Substituted-1, 3-benzothiazol-2-yl) benzene sulfonamide derivatives and evaluated for their *in vivo* anti-diabetic activity in a non-insulin-dependent diabetes mellitus rat model. Compounds (4) and (5) were found to be the most potent and docked into the crystal structure of 11β-HSD1.





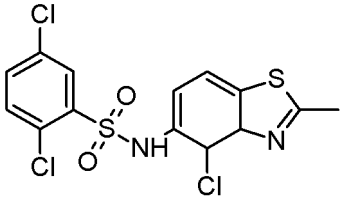
(4) (5)

Michael[4] *et al* (2004), designed a novel series of highly potent and selective (2-arylcarbamoyl-phenoxy)-acetic acid derivatives and screened for their *in vitro* enzyme inhibitory activity against aldose reductase for treatment of chronic diabetic complications. Among them, compound (6) had showed good inhibitory activity.



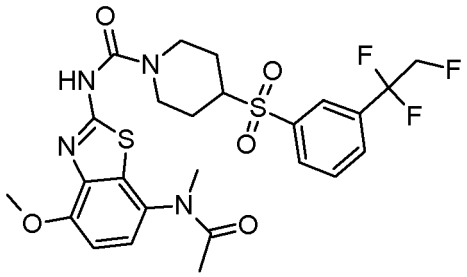
(6)

Xiangdong Su[5] *et al* (2006), discovered a series of novel Benzothiazole derivatives and their inhibitory activity against 11-HSD1 from human hepatic microsomes measured using a radioimmunoassay (RIA) method. The compound (7) showed good inhibitory activity.



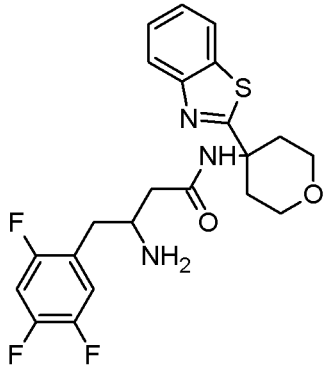
(7)

Fariborz Firooznia[6] *et al* (2011), synthesized N-(2-amino-4-methoxy-benzothiazol-7-yl)-N-ethyl-acetamide derivatives and evaluated for their selectivity against Adenosine A_{2B} and A_1 receptors for type 2 diabetes. Compound (8) showed an excellent selectivity against both receptors.



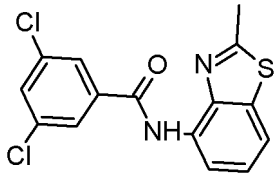
(8)

Aiko Nitta[7] *et al* (2008), a novel series of 3-amino-N-(4-aryl-1, 1-dioxothian-4-yl) butanamides and 3-amino-N-(4-aryltetrahydropyran-4-yl) butanamides were synthesized and evaluated as dipeptidyl peptidase IV (DPP-IV) inhibitors. Compound (9) showed good inhibitory activity.



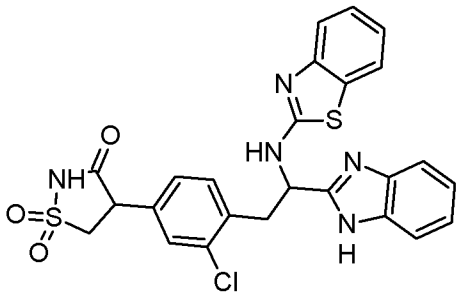
(9)

Nigel Vicker[8] *et al* (2007), discovered a novel Benzothiazole derivatives and screened for their activity against 11β-HSD1. Compound (10) showed good inhibitory activity.



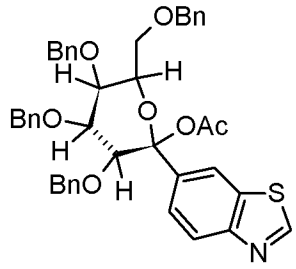
(10)

Richard[9] *et al* (2007), designed a Benzothiazole benzimidazole (S)-isothiazolidinone ((S)-IZD) derivatives through a peptidomimetic modification of the tripeptide (S)-IZD protein tyrosine phosphatase 1B (PTP1B) inhibitor. Among them, compound (11) showed good inhibitory activity.



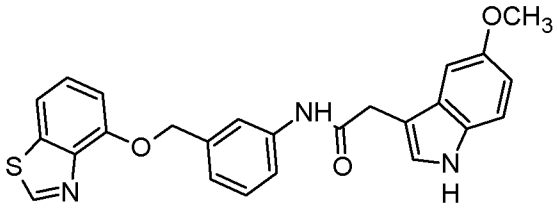
(11)

Alessandro Dondoni[10] *et al* (2003), synthesized a 2-lithiobenzothiazole fused to D-gluconolactone analogues and screened for their activity against glycosidase enzyme for type 2 diabetes. Among them, Compound (12) was the most potent.



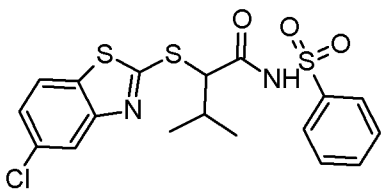
(12)

Guo Hua Jin[11] *et al* (2010), synthesized a series of Benzothiazole and evaluated their inhibitory activities for NO production in lipopolysaccharide-activated macrophages by the suppression of iNOS protein and mRNA expression. Among them, compound (13) was the most potent.



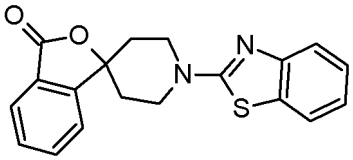
(13)

Alessandra Ammazzalorso[12] *et al* (2011), synthesized a new series of Benzothiazole based N-(phenylsulfonyl) amide analogues and evaluated for their selectivity against PPARα receptor. Compound (14) had showed good activity in this series.



(14)

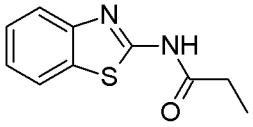
Yoshio Ogino[13] *et al* (2008), a novel class of 2-{3-oxospiro [isobenzofuran-1(3H), 40-piperidin]-10-yl} Benzothiazole analogues have synthesized and screened for their selectivity against NPY Y5 receptor. Among them, compound (15) had showed good activity.



(15)

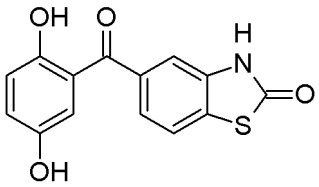
2.1.2 Antioxidant

Damien Cressier[14] *et al* (2009), discovered a new series of Thiadiazole and Benzothiazole derivatives and screened for their antioxidant activity by determining the DPPH and ABTS free radical scavenging using simple UV spectroscopic methods. Among them, compound (16) posses good activity.



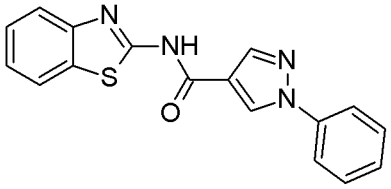
(16)

Tzvetomira Tzanova[15] *et al (*2009), designed a novel series of benzophenone fused Benzothiazole derivatives (17) and screened for their antioxidant activity by FRAP method.



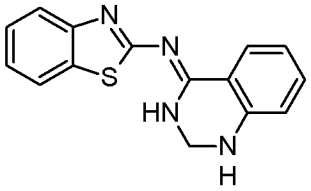
(17)

Samir bondock[16] *et al (*2009)**,** a poly functionally substituted heterocyclic incorporating Benzothiazole moiety was efficiently synthesized via its reactions with some N-nucleophiles. Representative compound (18) of the synthesized compounds was found to be a potent antimicrobial and antioxidant agents.



(18)

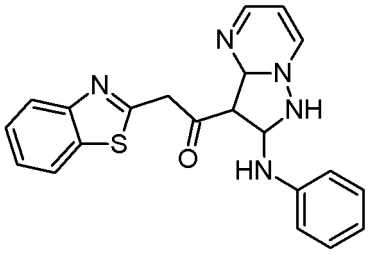
Meng-Chao Cui[17] *et al* (2010), a novel series of Schiff base derivatives of Benzothiazole were synthesized and screened for their antimicrobial and antioxidant activity by DPPH method. Among them, compound (19) was more potent.



(19)

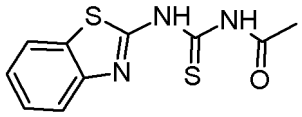
Antimicrobial

Samir Bondock[18] *et al* (2010)**,** discovered a new series of Thiazole, Thiophene, Pyrazole and other related products containing Benzothiazole moiety and screened for their antibacterial activity and antifungal. Among them, compound (20) was the most potent.



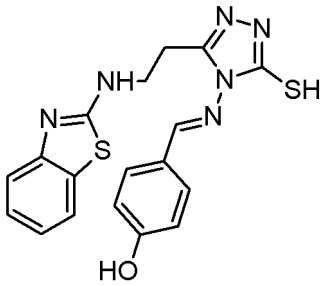
(20)

Sohail Saeed[19] *et al (*2010), synthesized a five series of thiourea derivatives bearing Benzothiazole moiety and evaluated for antimicrobial and anticancer activities. In preliminary MTT cytotoxicity studies, the thiourea derivative (21) was found to be the most potent.



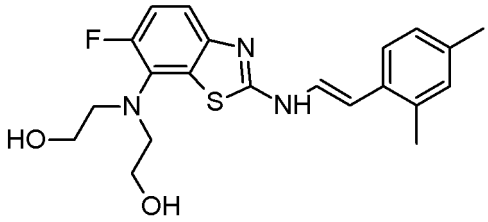
(21)

Balram Soni[20] *et al* (2010)**,** a novel series of Schiff bases of Benzothiazole derivatives were synthesized and screened for their antimicrobial activity. Compound (22) was more potent in this series.



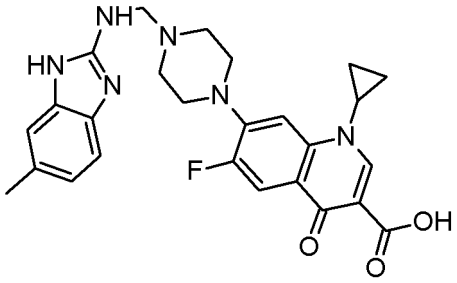
(22)

Barot Hitesh[21] *et al* (2010), synthesized 2-amino-7-chloro-6-fluoro Benzothiazole by using 4-fluoro-3-chloroaniline and potassium thiocyanate and screened for antibacterial (disc diffusion method) and antioxidant activity (ferric ion reduction method). Compound (23) shown promising activity.



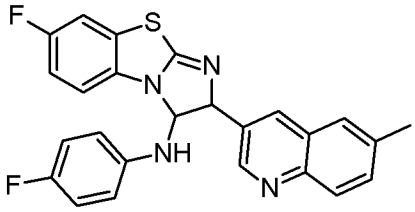
(23)

Prabodh Chander Sharma[22] *et al* (2011), a new series of fluoroquinolones annulated with 6-substituted-2-aminobenzothiazoles derivatives were synthesized and screened for their *in vitro* antibacterial activity against gram positive organism. Among them, compound (24) was the most potent.



(24)

Taleb[23] *et al* (2011), reported a imidazo[1,2-a]pyridine and imidazo[2,1-b][1,3]benzothiazole and evaluated as antimicrobial agents. Among them, compound (25) showed good activity as cefixime.

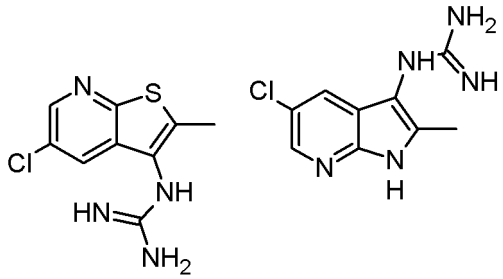


(25)

2.2 GUANIDINE DERIVATIVES

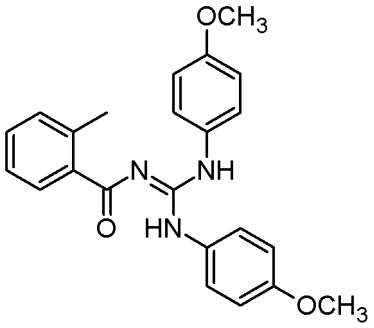
2.2.1 Anti-diabetic

Rajesh Bahekar[24] *et al* (2007), a series of substituted N-(thieno [2, 3-b] pyridin-3-yl)-guanidine) and N-(1H-pyrrolo [2, 3-b] pyridin-3-yl)-guanidine have synthesized and evauated *in vitro* glucose-dependent insulinotropic activity by using RIN5F (Rat Insulinoma cell) based assay. Among them, compound (26) was more potent.



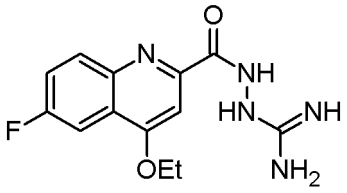
(26)

Silvio Cunha[25] *et al* (2001), synthesized N- benzoyl guanidine derivatives by converting N-benzoyl thiourea into guanidine by $HgCl_2$ method and evaluated for their anti-diabetic activity. Among them, compound (27) was more potent.



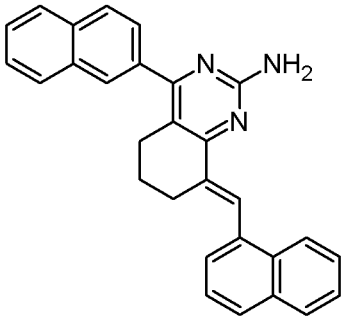
(27)

Dolore's Edmont[26] *et al* (2000), quinoline carboxyguanidine derivatives were synthesized by nucleophillic substitution reaction and evaluated for their *in vivo* anti- diabetic activity in a non-insulin-dependent diabetes mellitus rat model. Among them, compound (28) showed good activity.



(28)

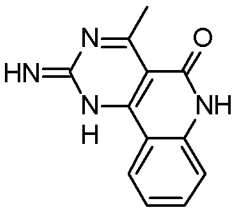
Nimisha Singh[27] *et al* (2011), synthesized a series of 8-(arylidene)-4-(aryl)-5, 6, 7, 8-tetrahydroquinazolin-2-ylamines and 9-(arylidene)-4-(aryl)-6, 7, 8, 9-tetrahydro-5H-cycloheptapyrimidin-2-ylamines by the reaction of bis-benzylidene cycloalkanones and guanidine hydrochloride in presence of NaH and evaluated against glycosidase and glycogen phosphorylase enzymes. Among them, compound (29) showed good activity.



(29)

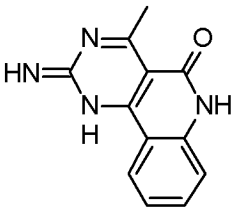
2.2.2 Antioxidant

Mathan Sankaran[28] *et al* (2010), a series of novel pyrimido and other fused quinoline derivatives were synthesized and screened for their *in vitro* antioxidant activity against radical scavenging capacity using DPPH and total antioxidant activity by FRAP. Among them, compound (30) shown promising antioxidant activity.



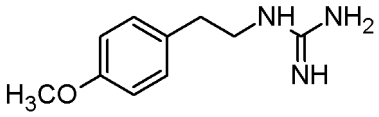
(30)

Jose Marquez[29] *et al* (2008), synthesized a cyclodextrin-derived thiourea and guanidine from taurine by the isothiocyanation reaction with thiophosgene in aqueous THF and evaluated for their antioxidant activity using DPPH method. Among them, compound (31) showed good activity.



(31)

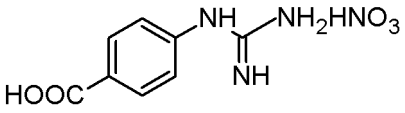
Maxime Mourer[30] *et al* (2009), para-guanidino ethyl phenol and its analogous were synthesized, fully characterized and evaluated as antibacterial agents and antioxidant agents. Among them, compound (32) showed good activity.



(32)

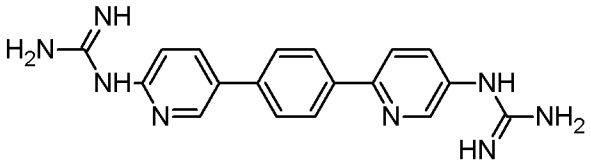
2.2.3 Antimicrobials

Karel Palat[31] *et al* (2004), a series of 4-substituted phenylguanidinium derivatives have synthesized and evaluated *in vitro* for antimicrobial activity by the broth micro dilution method against representative human pathogenic fungi. Among them, compound (33) showed good activity.



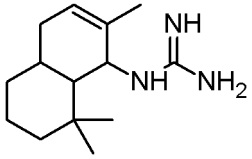
(33)

David Boykin[32] *et al* (2008), a series of triaryl guanidine and N-substituted guanidine designed to target the minor groove of DNA were synthesized and evaluated as antiprotozoal agents. Among them, compound (34) shown promising activity.



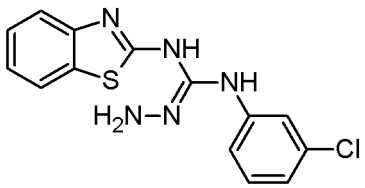
(34)

Miguel Zarraga[33] *et al (*2008), synthesized 11-guanidinodrimene (35) from drimenol and tested against *Candida albicans*.



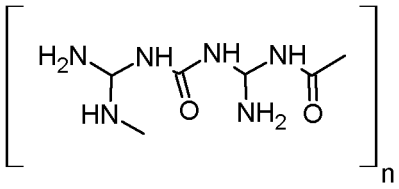
(35)

Ram Lakhan[34] *et al* (2002), synthesized fifteen new 1-aryl-2-amino-3-(4-arylthiazol-2-yl) (benzothiazol-2-yl) guanidine analogues and screened for their antimicrobial susceptibility by the standard disc diffusion method. Among them, compound (36) showed good activity.



(36)

Michel Lagrenee[35] *et al* (2008), synthesized a new corrosion inhibitor, namely, polyphosphate guanidine urea copolymer (PGUC). The results showed that the PGUC (37) had a broad inhibitory spectrum against bacteria.



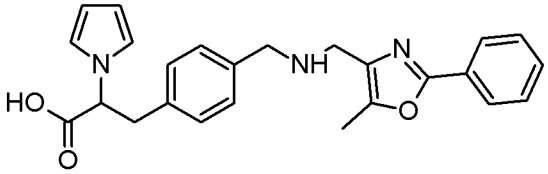
(37)

Huining Xiao[36] *et al* (2008), condensation and polymerization were used in the preparation of modified guanidine polymers. The two-step synthesis method increased the molecular weight of guanidine polymers. This was found to improve antimicrobial activity.

2.3 PROPANOIC ACID DERIVATIVES

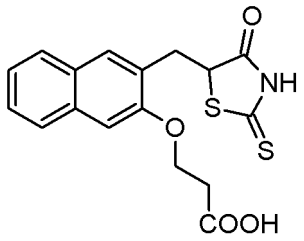
2.3.1 Anti-diabetic

Agustin Casimiro-Garcia[37] *et al* (2008), a new series of aryl or heteroaryl phenyl propanoic acid derivatives have synthesized and screened for their selectivity against PPRAα receptor for type 2 diabetes. Among them, compound (38) posses good activity.



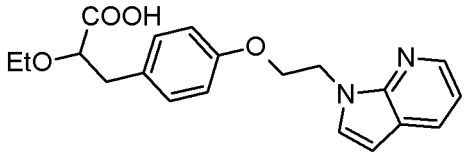
(38)

Makoto Murata[38] *et al (*1999), a new series of 5-[[2-(x-carboxyalkoxy) aryl] methylene]-4-oxo-2-thioxothiazolidine derivatives were synthesized and evaluated for their potency as aldose reductase inhibitors by *in vivo* and *in vitro.* The compound (*39*) shown promising inhibitory activity as standard (Zenarestat).



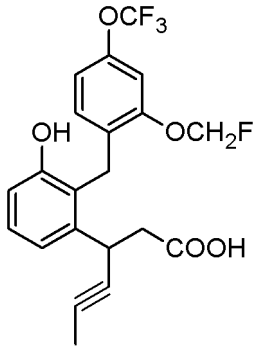
(39)

Zhefeng Cai[39] *et al* (2006), synthesized a series of azaindole-a-alkyloxy phenyl propionic acid analogues and evaluated for PPAR agonist activities. Structure activity relationship has developed for PPARα/β dual agonism. The compound (40) was identified as a potent, selective PPARα/β dual agonist.



(40)

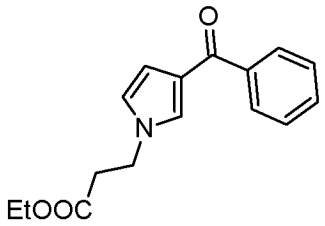
Shawn[40] *et al* (2011), a series of b-substituted 3-(4-aryloxyaryl) propanoic acids have prepared and evaluated for GPR40 agonists. The compound (41) shown promising activity by *in vivo* mouse mod



(41)

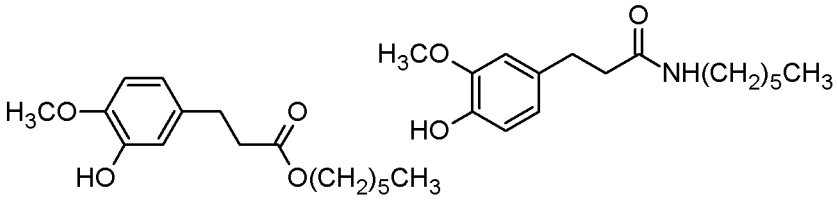
2.3.2 Antioxidant

Kyriaki Pegklidou[41] *et al* (2010), a series of Pyrrolyl-propionic and butyric-acid derivatives were synthesized and screened for their *in vitro* aldose reductase inhibitory activity and antioxidant activity against radical scavenging capacity using DPPH. Among them, compound (42) showed good activity.



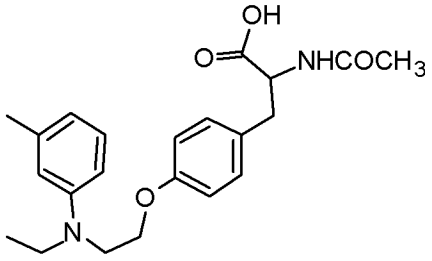
(42)

Fernanda[42] *et al* (2010), designed a lipophilic compounds structurally based on caffeic, hydrocaffeic, ferulic and hydroferulic acids and screened for their antioxidant activity as well as their partition coefficients and redox potentials. Among them, compound (43) showed good activity.



(43)

Rakesh Kumar[43] *et al* (2007), reported a series of N-acetyl-L-tyrosine derivatives and screened for their *in vitro* PPAR agonist and antioxidant activity by DPPH. Among them, compound (44) shown promising activity.

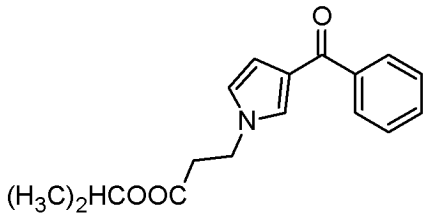


(44)

Barbara Morzyk-Ociepa[44] *et al* (2008), reported novel catena-poly[(di-m3-aqua)(h2-:-m2-indole-3-propionato-O)(m3-indole-3-propionato-O)disodium],[Na2(I3PA)2(H2O)2] characterized by X-ray diffraction analysis and infrared and Raman spectroscopic methods and screened for their biological activity.

2.3.3 Antimicrobials

Kyriaki Pegklidou[45] *et al* (20O9), a series of Pyrrolyl-propionic and butyric-acid derivatives were synthesized and screened for their *in vitro* antibacterial activity by disc diffusion method. Among them, compound (45) had showed good activity.



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Chapter 3

Methodology

METHODOLOGY

3. 1. RESEARCH ENVISAGED

Benzothiazole is a versatile heterocyclic compound and shows varied pharmacological activities like Anti-tumer[1], Anti-diabetic[2], Anti-tubercular[3], Anti-inflammatory[4], Analgesic[5] and Anti-microbial[6].

Diabetes[7] around the world and is one of the major public health challenges of the 21st century. The number of cases worldwide in 2000 is estimated to be about 171 million and its rise to 366 million in 2030. The World Health Organization (WHO) estimated that, diabetes-related deaths will increase by more than 50% in the next 10 decades. Especially in upper-middle income countries, diabetes deaths are projected to increase by over 80% between 2006 and 2015. This circumstance results that the demand for medical care in type 2 diabetes will continue to increase.

Microbial infections are gaining more attention towards the medicinal chemist. Since most of the currently used antibacterials or antibiotics are resistance to the vast of the microbes. Hence there is a need to develop a newer antimicrobial agent with multi target to combat the problem of resistance.

Over production of reactive oxygen and nitrogen species (ROS/RNS) leads to lowered antioxidant defense and alterations of enzymatic pathways. This contribute to endothelial, vascular and neurovascular dysfunction. Over the past decade, there has been substantial interest in oxidative stress and its potential role in diabetogenesis, development of diabetic complications, atherosclerosis and associated cardiovascular disease. So developing compounds containing both anti-oxidant and anti-diabetic activity is a relevant adjuvant pharmacotherapy.

Keeping on the above consideration and based on literature we synthesize some novel analogue.

- Synthesis of some novel Benzothiazole derivatives bearing Guanido group.
- The linker group (methenyl and phenyl) have been introduced between Benzothiazole and Guanidinopropionic ester.
- Characterization of synthesized compounds by various analytical techniques like TLC, FTIR, H^1NMR and Mass Spectral studies.

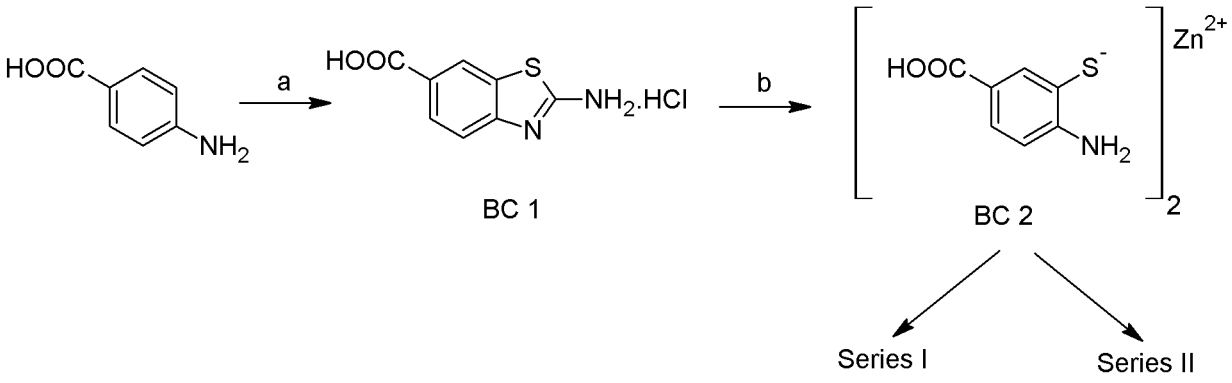
BIOLOGICAL SCREENING

- *In vitro* Aldose Reductase enzyme inhibition assay technique.
- Screening for antibacterial activity against bacterial organisms like *Micrococcus luteus, Staphylococcus aureus, Bacillus subtilies, Cornybacterium diphtheria, Bacillus linctus, Escherichia coli, Pseudomonas aureginosa, Rhodosporum rubrum and Vibrio cholera* by Disc diffusion method and Minimum inhibitory concentration (MIC) by serial dilution method.
- Screening for antifungal activity against fungal organisms like *Candida albicans, Aspergillus niger, Aspergillus fumigates, Aspergillus parasites* by Disc Diffusion method and Minimum inhibitory concentration (MIC) by serial dilution method.
- Screening for antioxidant activity by using *in vitro* DPPH method.

3.2. PLAN OF STUDY

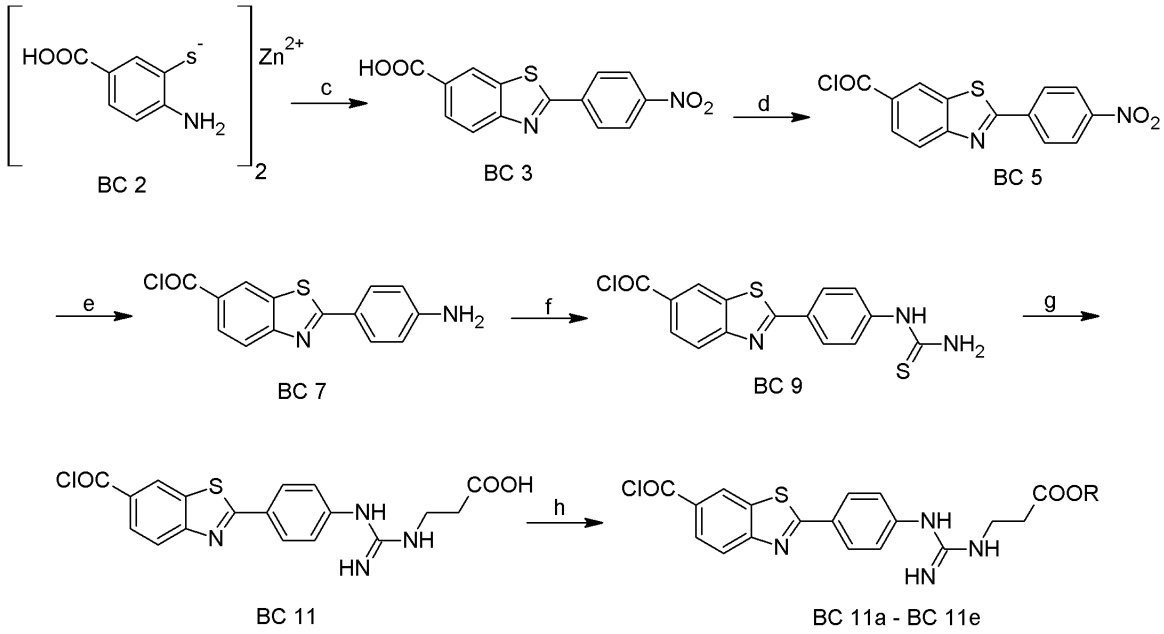
Phase I

Scheme



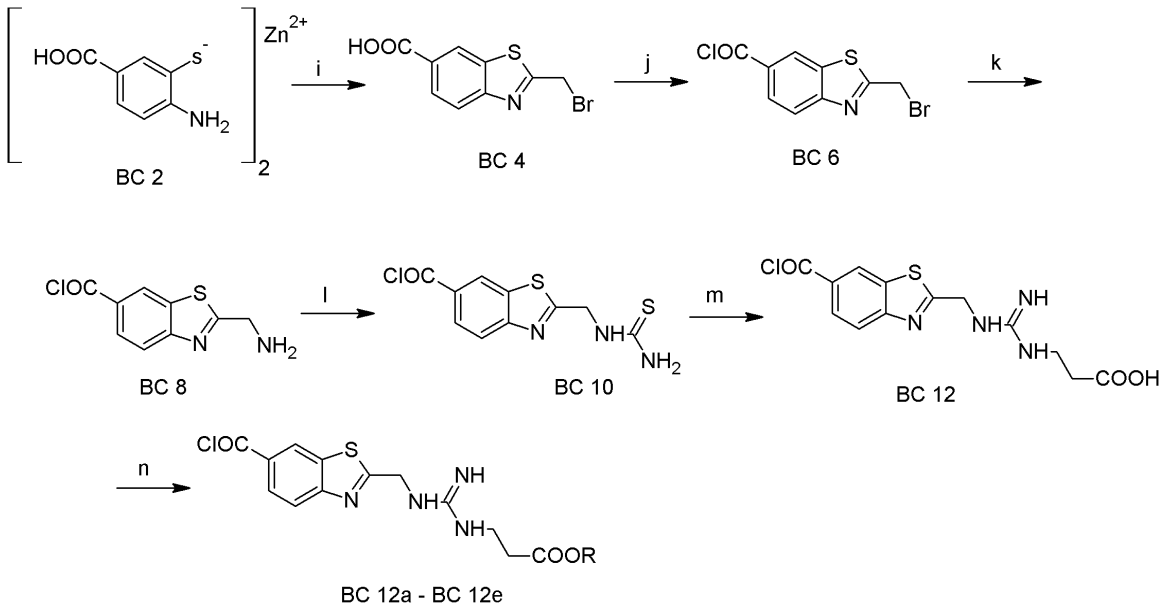
Reagents & conditions: (a) $NaSCN/Br_2$, HCl (stir for 2 hrs at 5^0c); (b) KOH, $ZnCl_2$, H_2O (reflux for 4 hrs).

Series I



Reagents & conditions : (c) *p*- nitro benzoylchloride, C_6H_5N (stir for 1 hr at 80^0c); (d) $SOCl_2$ (reflux for1 hr); (e) $SnCl_2$, C_2H_5OH, HCl (heat in water bath for 6 hrs); (f) 50% NH_4SCN, HCl (heat on steam bath for 3-4hrs); (g) silica gelG, $CuSO_4.5H_2O$, β alanine, TEA, THF (stir for 5 hrs); (h) ROH, H_2SO_4 (reflux for 6 hrs).

Series II



Reagents & conditions: (i) Br-CH_2-CO-Br, C_6H_5N (stir for 2 hrs at 80^0c); (j) $SOCl_2$ (reflux for 90 mts); (k) NH_3 (heat in water bath for 1 hr); (l) 50% NH_4SCN, HCl (heat on steam bath for 5-6 hrs); (m) silicagel G, $CuSO_4.5H_2O$, β alanine, TEA, THF (stir for 7 hrs); (n) ROH, con.H_2SO_4 (stir for 7 hrs).

Phase II

Characterization: All the newly synthesized compounds will be characterized by Melting point determination, Solubility property, TLC analysis and their structure will be elucidated by IR- Spectroscopy, H^1 NMR Spectroscopy and MASS Spectroscopy.

Phase III

Biological evaluation:

***In vitro* Aldose reductase enzyme inhibition assay technique:** All the synthesized compounds will be screened for *in-vitro* Aldose reductase enzyme inhibition assay technique.

***In vitro* antimicrobial activity**: All the synthesized compounds will be screened for antimicrobial activity against various bacteria and fungi by using Disc Diffusion method and Minimum Inhibitory Concentration (MIC) method.

***In vitro* antioxidant activity:** All the synthesized compounds will be evaluated for *in vitro* antioxidant activity by DPPH method.

EXPERIMENTAL

General Procedure

Synthesis of 2-aminobenzothiazole-6-carboxylic acid (BC 1)[8]

Sodium thiocyanate (65g, 0.8 mol) was added to a suspension of 4-amino-benzoic acid (1, 100g, 0.8 mol) in methanol followed by the addition of bromine (38ml, 0.8 mol) in portions. The above solutions was allowed to cool at -10^0C and stirred for 2 hrs while keeping the inner temperature below -5^0C. The precipitate was then filtered and suspended in 350 ml of 1M hydrochloric acid and refluxed for 30 min. After immediate filtration, 150 ml concentrated hydrochloric acid was added to the hot filtrate to yield a white precipitate of 2-aminobenzothiazole-6-carboxylic acid (87g, 75%, m.p: 202^0C, R_f = 0.7 ($CHCl_3$:CH_3OH 1:1)).

Synthesis of Zinc salt of 4-amino-3-mercaptobenzoic acid (BC 2)

Compound 2-aminobenzothiazole-6-carboxylic acid (BC 1) (9.18g, 40 mmol) was dissolved in a potassium hydroxide solution (45g KOH\45 ml water) and refluxed for 3 hrs. After being cooled to room temperature, the solution was neutralized by concentrated hydrochloric acid and aqueous solution of Zinc chloride (0.02mmol in 25 ml of water) was added slowly while white solid precipitated out. The suspension was acidified by glacial acetic acid. The solid was filtered, washed with water, and dried in a vacuum to yield a white precipitate of 4-amino-3-mercaptobenzoic acid (11g, 26%, m.p: 184^0C, R_f = 0.78 ($CHCl_3$:CH_3OH 1:1)).

Series I

Synthesis of 2-(4'-nitrophenyl)-6-(benzothiazolyl) carboxylicacid (BC 3)

Compound 4-amino-3-mercaptobenzoic acid (BC 2) (8.18g, 20mmol) was suspended in pyridine (50 ml) and heated to 80^0C. *p*-nitrobenzoyl chloride (8g, 42.8 mmol) was added to that suspension and stirred for 1 hour. After being cooled to room temperature, the precipitate was filtered, washed with dilute hydrochloric acid and water, and dried under

vacuum to yield 2-(4'-nitrophenyl)-6-(benzothiazolyl) carboxylic acid (7g, 85%, m.p: 104^0C, R_f = 0.72 ($CHCl_3$:CH_3OH 1:1)).

Synthesis of 2-(4-nitro-phenyl)-benzothiazole-6-carbonyl chloride (BC 5)

Compound 2-(4'-nitrophenyl)-6-(benzothiazolyl) carboxylic acid (BC 3) (1g, 3.3 mmol) was suspended in thionyl chloride (5 ml) and refluxed for 1 hr and excess thionyl chloride was evaporated under reduced pressure to yield 2-(4-nitro-phenyl)-benzothiazole-6-carbonyl chloride. (0.9g, 95%, m.p: 117^0C, R_f = 0.81 ($CHCl_3$:CH_3OH 1:1)).

Synthesis of 2-(4-amino-phenyl)-benzothiazole-6-carbonyl chloride (BC 7)

Compound 2-(4-nitro-phenyl)-benzothiazole-6-carbonyl chloride (BC 5) (7.5g, 0.02mol) was dissolved in concentrated hydrochloric acid (13 ml) and ethanol (100 ml) followed tin chloride (2g, 10 mmol) was added to that suspension and heated to 80 ^{0}C for 5 hrs. After being cooled to room temperature, the solid was filtered, washed with concentrated hydrochloric acid , water and dilute ammonium and dried in vacuum to get 2-(4-amino-phenyl)-benzothiazole-6-carbonyl chloride. (6g, 88%, m.p: 137^0C, R_f = 0.7 ($CHCl_3$:CH_3OH 1:1)).

Synthesis of 2-[4-(carbamothioylamino) phenyl]-1, 3-benzothiazole-6-carboxylic acid (BC 9)[9]

Compound 2-(4-amino-phenyl)-benzothiazole-6-carbonyl chloride (BC 7) (0.02 mol, 5.78g) was dissolved in concentrated hydrochloric acid (25 ml) and warmed. A saturated solution of ammonium thiocyanate (saturated, 50%) in water was added slowly in above solution, the mixture was boiled for 3-4 hrs until the solution turns turbid. The turbid solution was poured in cold water. The separated phenylthiourea precipitate was filtered and crystallized with aqueous ethanol. (7g, 97%, m.p: 128^0C, R_f = 0.6 (Toluene: Ethylaetate1:2))

Synthesis of 2-{4-[*N*'-(2-carboxyethyl) carbamimidamido] phenyl}-1, 3-benzothiazole-6-carboxylic acid (BC 11)[10]

β alanine (0.02 mol, 1.78g) was added to the stirring mixture of compound 2-[4-(carbamothioylamino)phenyl]-1,3-benzothiazole-6-carboxylic acid (BC 9) (0.02 mol, 6.94g) containing silica gel G (0.01g), cupric sulphate pentahydate (0.01g), triethylamine (2 ml) in tetrahydrofuran (25 ml). Stirring was continued for 5 hrs and an excess of solvent was evaporated at room temperature and recrystallized with methanol. (3g, 40%, m.p: 167^0C, R_f = 0.8 (Toluene: Ethylaetate1:2)).

Synthesis of ester derivative of 2-{4-[*N*'-(2-carboxyethyl) carbamimidamido] phenyl}-1, 3-benzothiazole-6-carboxylic acid (BC 11a – BC 11e) [11]

Compound BC 11 2-{4-[*N*'-(2-carboxyethyl)carbamimidamido]phenyl}-1,3-benzothiazole-6-carboxylic acid (0.001mol, 0.3g) was dissolved in respective alcohol derivatives (a-ethyl, b-n-butyl, c-iso propyl, d-phenyl, e-tolyl) and an appropriate quantity of concentrated sulphuric acid (2 ml) was added and refluxed for 5-6 hrs. The reaction completion was monitored by thin layer chromatography.

Series II

Synthesis of 2-(bromo methyl)-benzothiazole-carboxylic acid (BC 4)

Compound 4-amino-3-mercaptobenzoic acid (BC 2) (8.18, 20mmol) was suspended in pyridine (50 ml) and heated to 80 ^{0}C and then bromoacetylbromide (3.7 ml, 42.8 mmol) was added in portions to get a clear solution and stirred for 2 hrs. After being cooled to room temperature, the precipitate was filtered, washed with dilute hydrochloric acid and water, and dried under vacuum to afford 2-(bromo methyl)-6-(benzothiazolyl) carboxylic acid. (6g, 55%, m.p: 140^0C, R_f = 0.7 ($CHCl_3$:CH_3OH 1:1)).

Synthesis of 2-(bromo methyl)-benzothiazole-6-carbonyl chloride (BC 6)

Compound 2-(bromo methyl)-6-(benzothiazolyl) benzothiazole (BC 4) (0.8g, 3.3 mmol) was suspended in thionyl chloride (5 ml) and refluxed for 90 mts. Then excess thionyl chloride was evaporated under reduced pressure to get 2-(bromo methyl)-benzothiazole-6-carbonyl chloride. (0.6g, 75%, m.p: 138^0C, R_f = 0.9 ($CHCl_3$:CH_3OH 1:1)).

Synthesis of 2-(amino methyl)-benzothiazole-6-carbonyl chloride (BC 8)[12]

To compound 2-(bromo methyl)-benzothiazole-6-carbonyl chloride (BC 6) (2.8g, 0.01mol) was suspended in ammonia (30 ml) and boiled for 1 hr. After being cooled to room temperature, the solid was filtered, and dried in a vacuum to yield 2-(amino methyl)-benzothiazole-6-carbonyl chloride. (2g, 71%, m.p: 105^0C, R_f = 0.65 ($CHCl_3$:CH_3OH 1:1)).

Synthesis of 2-[4-(carbamothioylamino) methyl]-1, 3-benzothiazole-6-carboxylic acid (BC 10)

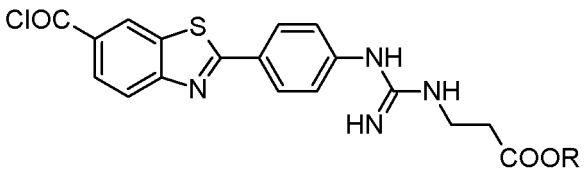
Compound 2-(amino methyl)-benzothiazole-6-carbonyl chloride (BC 8) (0.02 mol, 4.54g) was dissolved in concentrated hydrochloric acid (25 ml) and warmed. A saturated solution of ammonium thiocyanate (saturated, 50%) in water was added slowly in above solution, the mixture was boiled for 5-6 hrs until the solution turns turbid. The turbid solution was poured in cold water. The separated thiourea precipitate was filtered and crystallized with aqueous ethanol. (4g, 88%, m.p: 119^0C, R_f = 0.73 (Toluene: Ethylaetate 1:2)).

Synthesis of 2-{4-[*N*'-(2-carboxyethyl) carbamimidamido] methyl}-1,3-benzothiazole-6-carboxylic acid (BC 12)

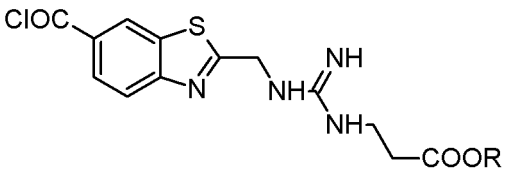
β alanine (0.02 mol, 1.78g) was added to the stirring mixture of compound 2-[4-(carbamothioylamino)methyl]-1,3-benzothiazole-6-carboxylic acid (BC 10) (0.02 mol, 5.8g) containing silica gel G (0.01g), cupric sulphate pentahydate (0.01g), triethyl amine (2 ml) in tetrahydrofuran (25 ml). Stirring was continued for 6 hrs and an excess of solvent was evaporated at room temperature and recrystallized with aqueous methanol. (2.5g, 40%, m.p: 134^0C, R_f = 0.8 (Toluene: Ethylaetate 1:2)).

Synthesis of ester derivative of 2-{4-[*N*'-(2-carboxyethyl) carbamimidamido] methyl}-1, 3-benzothiazole-6-carboxylic acid (BC 12a- BC 12e)

Compound 2-{4-[*N*'-(2-carboxyethyl) carbamimidamido] methyl}-1, 3-benzothiazole-6-carboxylic acid (BC 12) (0.001mol, 0.3g) was dissolved in respective alcohol derivatives (a-ethyl, b-n-butyl, c-iso propyl, d-phenyl, e-tolyl) and an appropriate quantity of concentrated sulphuric acid (2 ml) was added and refluxed for 5-6 hrs. The reaction completion was monitored by thin layer chromatography.

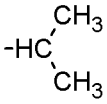
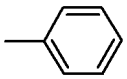
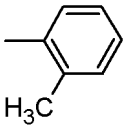
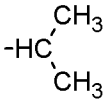
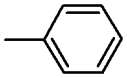
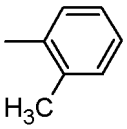


(BC 11a – BC 11e)



(BC 12a – BC 12e)

Table 1: Physical data of synthesized compounds

Code	R	M.F	M.W	M.P(^{0}C)	Yield (%)	Clogp
BC 11a	C_2H_5	$C_{20}H_{19}O_3N_4SCl$	430.90	190	62	3.051
BC 11b	C_4H_9	$C_{22}H_{23}O_3N_4SCl$	458.96	170	58	4.120
BC 11c		$C_{21}H_{21}O_3N_4SCl$	444.93	145	51	3.360
BC 11d		$C_{24}H_{19}O_3N_4SCl$	478.95	*	67	3.761
BC 11e		$C_{25}H_{21}O_3N_4SCl$	492.97	*	75	4.260
BC 12a	C_2H_5	$C_{15}H_{17}O_3N_4SCl$	368.83	95	64	1.337
BC 12b	C_4H_9	$C_{17}H_{21}O_3N_4SCl$	396.89	115	57	2.396
BC 12c		$C_{16}H_{19}O_3N_4SCl$	382.86	130	67	1.647
BC 12d		$C_{19}H_{17}O_3N_4SCl$	416.88	*	78	2.047
BC 12e		$C_{20}H_{19}O_3N_4SCl$	430.90	*	70	2.546

* Compounds are liquid in state

All compounds are soluble in DMSO

3.3. ANALYTICAL WORK

THE LAYER CHROMATOGRAPHY

Thin layer Chromatography is a method of analysis in which the stationary phase, a finely divided solid, is spread as a thin layer on a rigid supporting plate and the mobile phase, a liquid is allowed to migrate across the surface of the plate.

Applications of TLC

1. To establish the purity and authenticity of starting materials and reagents.
2. To monitor the reactions, particularly in the case of new reactions.
3. Assessment of purity of a crude reaction product.
4. The optimum of experimental conditions to achieve the highest possible yield of product.

Provided that the experimental conditions are reproducible, the movement of any substance relative to the solvent front in a given chromatographic system is constant and characteristic of the substance. The constant is called as retardation factors (R_f) and is defined as

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance traveled by the solvent front}}$$

True reproducibility in R_f value is, however, rarely achieved in practice due to minor changes in a number of variables such as:

a. The particle size of different batches of adsorbent.
b. The solvent composition and the degree of saturation of the chamber atmosphere with solvent vapor.
c. Prior activation and storage conditions of the plates.
d. The thickness of adsorbent layer, etc.

It is therefore, not desirable to use R_f value in isolation as a criteria for identity.

TLC were performed as following procedure

Dimension of plates	:	5*20cm
Stationary phase	:	Silica gel-G 9E-merck
Mobile phase	:	Chloroform: Methanol (1:1)
Technique	:	Ascending
Detection Method	:	UV chamber at 254nm

Preparation of Plates

Uniform slurry of silica get G was prepared by addition of distilled water. This was then poured into a spreading through and drawn across a series of glass plates of size 5*20cm, depositing a uniform layer of stationary phase of 0.25mm thickness. The plates were air dried and then activated by heating at 110 ^{0}C for one hour. The plates were stored over desiccators until used.

Mobile Phase

Evaluation of various mobile phases was tried alone or in combination for each compound in which Chloroform: Methanol (1:1) was found to be suited.

Sample Application and Development

The samples were applied as small spot at about 2cm form the base of the plate. For ascending development of the thin layer chromatogram, the plate was placed in a TLC Chamber, which was saturated with mobile phase containing the developing solvent to a depth of about 0.5cm. The solvent was allowed to move up the plate until it travelled a distance of about 15cm from the point of the sample, on a 20cm plate. The plate was then removed from the chamber, the solvent front was marked by scratching the surface and the plate was allowed to be evaporated.

Detection

After the chromatogram was developed the solute sports need to be made visible in order to determine their R_f values. UV chamber was employed for the detection of the compounds by placing the plate in a chamber at short wavelength (254nm).The solute was visible as blue spot.

Table 2: R_f value of synthesized compounds

S.NO	Compound	R_f value
1	BC 11a	0.91
2	BC 11b	0.73
3	BC 11c	0.84
4	BC 11d	0.88
5	BC 11e	0.90
6	BC 12a	0.80
7	BC 12b	0.78
8	BC 12c	0.81
9	BC 12d	0.79
10	BC 12e	0.84

3.4. INFRARED SPECTRAL STUDY

The range of electromagnetic radiation between 0.8 and 500µm is referred as infrared radiation. The IR spectrum is represented with percent transmittance as the ordinate and the wave number (cm-1) as the abscissa. The most commonly used region of IR spectrum in pharmaceutical chemistry is between 2.5µm (400cm-1) and 16µm (625cm-1). Two major applications of IR spectrometry are characterization of various molecules includes Determination of identity of a compound by means of spectral comparison with that of authentic sample.

Verification of the presence of functional groups in unknown molecules which is quite important in the structural elucidation of synthetic organic compounds or substances isolated from natural sources. Samples were prepared by KBr Disc method. Solid samples (0.5-1.Omg) were intimately mixed with powdered potassium bromide. Mixing was effected through grinding in a smooth agate mortar and the mixture pressed between a punch and disc under the presence of 1, 00,000-15,000 psi into a transparent disk. The infrared spectral study was done on JASCO FTIR 4100. All absorption values are expressed in wave numbers cm-1

The spectral data are given as follows.

Table-3

Code	Absorbance(cm^{-1})	Groups
BC 1	3400.01	Primary amine -N-H stretch
	3127.01	Carboxylic acid -O-H stretch
	1706.07	Carboxylic acid -C=O stretch
	1605.45	Aromatic -C=C- ring stretch
	1635.07	Cyclic imino -C=N- stretch
	670.14	Thiol -C-S stretch
BC 2	3300.02	Primary amine -N-H stretch
	3127.01	Carboxylic acid -O-H stretch
	1706.69	Carboxylic acid -C=O stretch
	1609.31	Aromatic -C=C- ring stretch
	1247.72	Primary amine -C-N- stretch
BC 3	3118.33	Carboxylic acid -O-H stretch

	1691.67	Carboxylic acid -C=O stretch
	1603.53	Aromatic -C=C- ring stretch
	1523.31	Aromatic –N=O stretch
BC 4	3118.33	Carboxylic acid -O-H stretch
	2895.07	Methylene -C-H stretch
	1691.67	Carboxylic acid -C=O stretch
	1525.65	Aromatic -C=C- ring stretch
	1150.02	CH_2X, -C-H stretch
	700.29	Aliphatic -C-Br stretch
BC 5	1800.01	Acid chloride -C=O stretch
	1609.67	Aromatic -C=C- ring stretch
	1501.03	Aromatic -N=O stretch
	1376.55	Aromatic -N=O stretch
	850.02	Acid chloride -C-Cl stretch
BC 6	2895.07	Methylene -C-H stretch
	1800.01	Acid chloride -C=O stretch
	1602.56	Aromatic -C=C- ring stretch
	850.03	Acid chloride -C-Cl stretch
	700.14	Aliphatic -C-Br stretch
	670.14	Thiol -C-S stretch
BC 7	3127.97	Primary amine -N-H stretch
	1603.52	Aromatic -C=C- stretch
	850.03	Acid chloride -C-Cl stretch
	670.14	Thiol -C-S stretch
BC 8	3127.97	Primary amine -N-H stretch
	2895.07	Methylene -C-H stretch
	850.03	Acid chloride -C-Cl stretch
	669.17	Thiol -C-S stretch
BC 9	3127.97	Primary amine -N-H stretch
	2073.16	R-N=C=S stretch
	1600.63	Aromatic -C=C- in ring stretch
	1322.93	Ar-N stretch
	670.14	Thiol -C-S stretch
BC 10	3135.69	Primary amine -N-H stretch
	2895.03	Methylene -C-H stretch
	2073.16	R-N=C=S stretch
	1618.95	R-N-H stretch
	1546.63	Disulfide -C=S stretch
	1262.18	Aliphatic amine -C-N stretch

	668.21	Thiol -C-S- stretch
BC 11	3353.61	Secondary amine -N-H stretch
	3125.08	Carboxylic acid -O-H stretch
	2890.01	Methylene -C-H stretch
	1723.01	Carboxylic acid-C=O stretch
	1630.04	Imino -C=N- stretch
	1619.91	Aromatic -C=C- in ring stretch
	1400.07	Methylene -C-H bend
BC 12	3350.71	Secondary amine -N-H stretch
	3127.01	Carboxylic acid -O-H stretch
	1725.98	Carboxylic acid -C=O stretch
	1640.01	Imino -C=N- stretch
	1596.77	Aromatic -C=C- in ring stretch
	1400.07	Methylene -C-H bend
BC 11a	3300.01	Secondary amine -N-H stretch
	2953.01	Methyl -C-H stretch
	2853.03	Methylene -C-H stretch
	1800.00	Acid chloride -C=O stretch
	1750.01	Aliphatic ester -C=O stretch
	1600.04	Aromatic -C=C- ring stretch
BC 11b	3354.44	Secondary amine -N-H stretch
	2950.04	Methyl -C-H stretch
	2800.01	Methylene -C-H stretch
	1750.01	Aliphatic ester -C=O stretch
	1600.02	Aromatic -C=C- ring stretch
	720.01	Straight chain alkane
BC 11c	3347.57	Secondary amine -N-H stretch
	1700.02	Aliphatic ester -C=O stretch
	1600.03	Aromatic -C=C- ring stretch
	1425.04	Methyl -C-H bend
	1380.01	Isopropyl group
BC 11d	3122.19	Secondary amine -N-H stretch
	1770.01	Phenol ester -C=O stretch
	1650.77	Imino -C=N- stretch
	1519.63	Aromatic -C=C- stretch
BC 11e	3161.72	Secondary amine -N-H stretch
	1776.65	Esters -C=O stretch
	1632.45	Imino -C=N- stretch
	1610.01	Aromatic -C=C- ring stretch

	1422.01	Methyl -C-H stretch
BC 12a	3353.36	Secondary amine -N-H stretch
	2956.01	Methyl -C-H stretch
	2850.88	Methylene -C-H stretch
	1750.02	Aliphatic ester -C=O stretch
	1600.01	Aromatic -C=C- ring stretch
BC 12b	3383.26	Secondary amine -N-H stretch
	2950.03	Methyl -C-H stretch
	2850.01	Methylene -C-H stretch
	1700.02	Aliphatic ester -C=O stretch
	720.03	Straight chain alkyl group
BC 12c	3359.14	Secondary amine -N-H stretch
	2956.01	Methyl -C-H stretch
	2849.92	Methylene -C-H stretch
	1750.02	Aliphatic ester -C=O stretch
	1450.03	Methyl -C-H bend
	1350.01	Isopropyl group
BC 12d	3135.69	Secondary amine -N-H stretch
	1720.01	Esters -C=O stretch
	1650.77	Imino -C=N- stretch
	1618.95	Aromatic -C=C- ring stretch
BC 12e	3375.18	Secondary amine -N-H stretch
	1776.65	Esters -C=O stretch
	1632.45	Imino -C=N- stretch
	1610.01	Aromatic -C=C- ring stretch
	1422.01	Methyl -C-H stretch

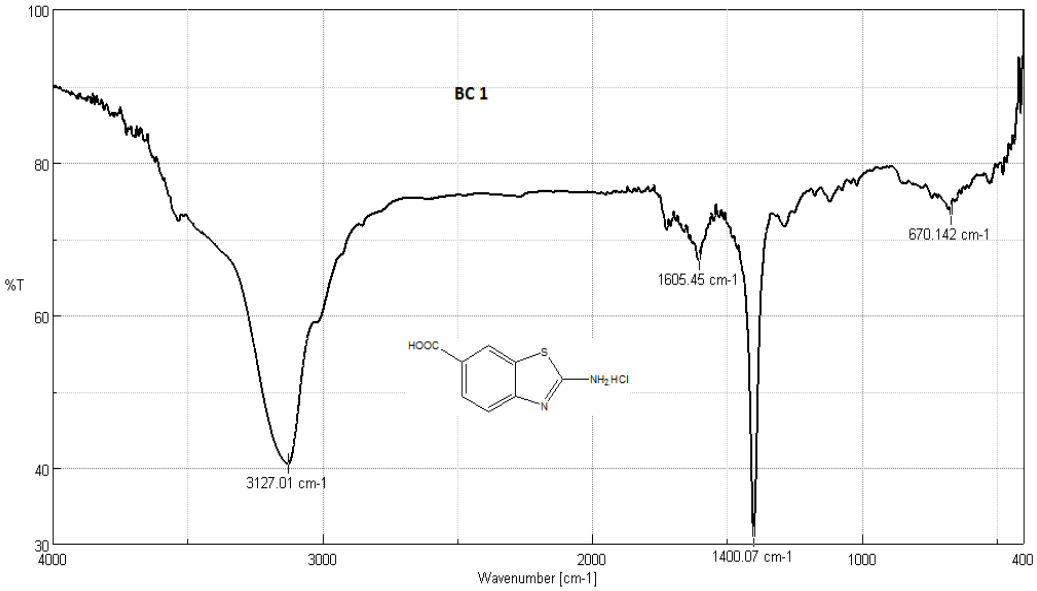


Fig 1: IR spectrum of compound BC 1

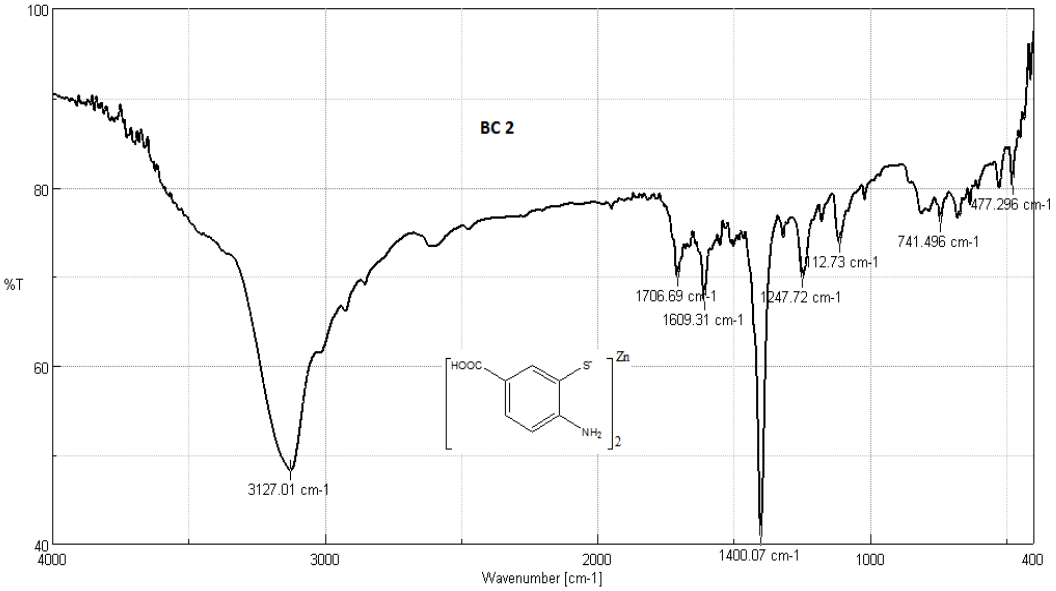


Fig 2: IR spectrum of compound BC 2

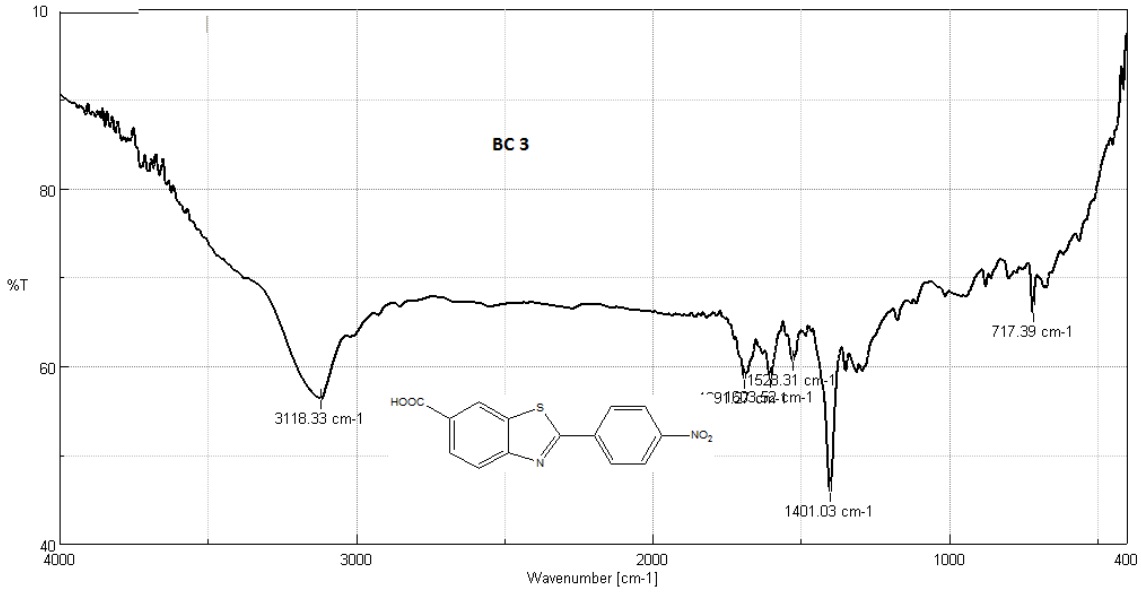


Fig 3: IR spectrum of compound BC 3

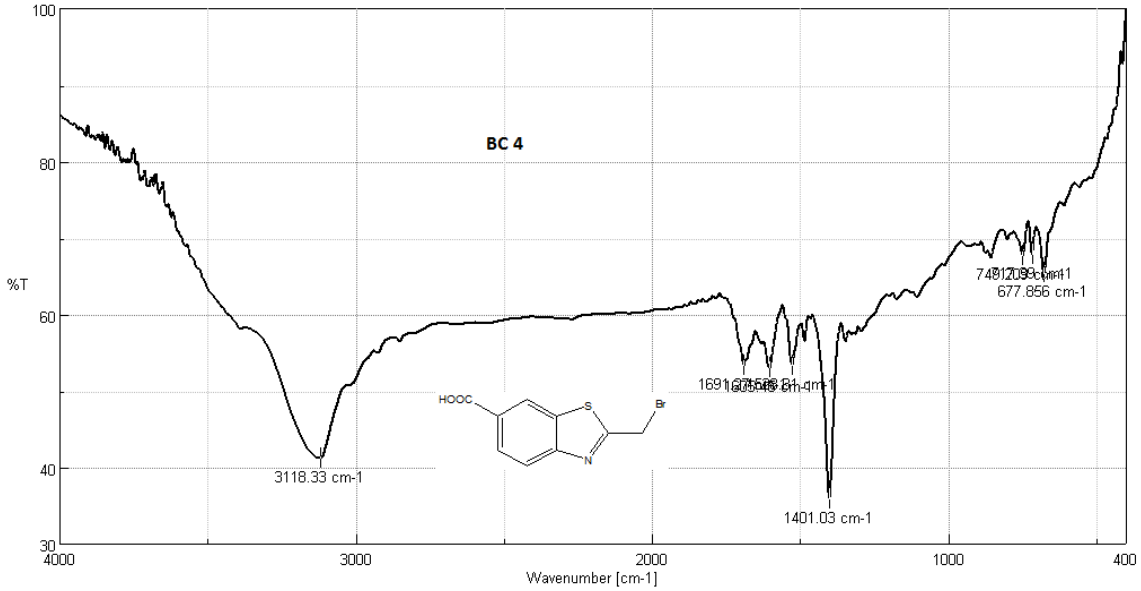


Fig 4: IR spectrum of compound BC 4

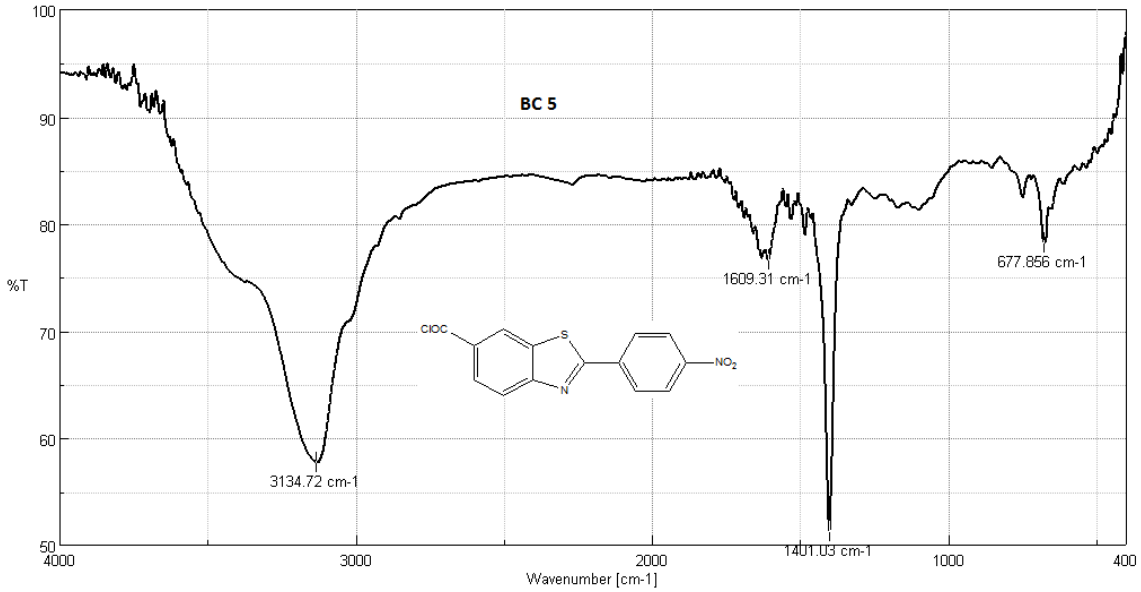


Fig 5: IR spectrum of compound BC 5

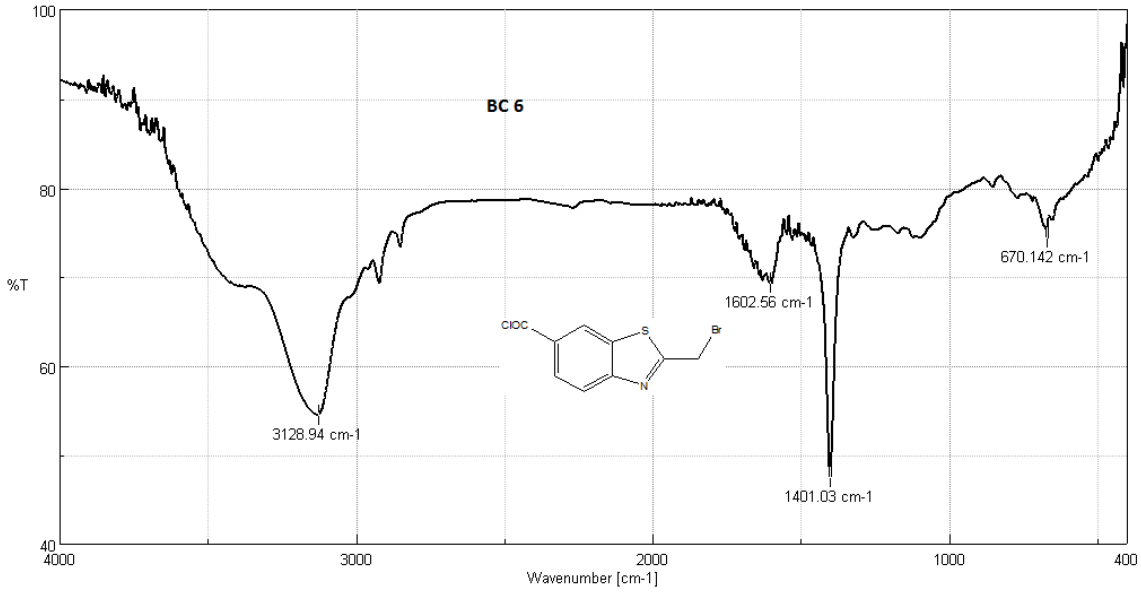


Fig 6: IR spectrum of compound BC 6

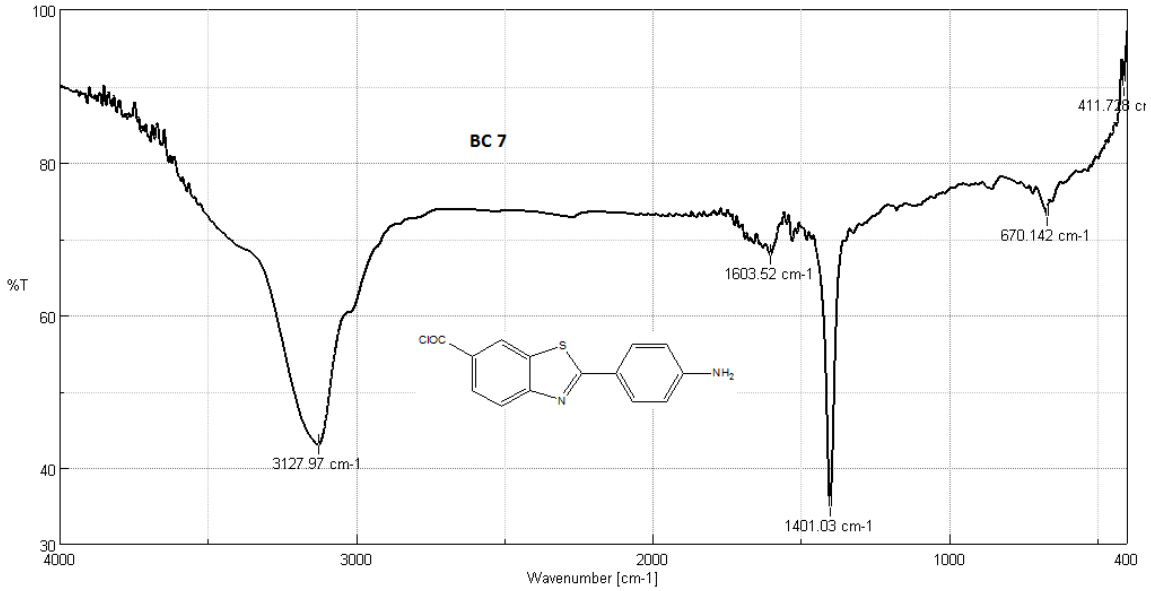


Fig 7: IR spectrum of compound BC 7

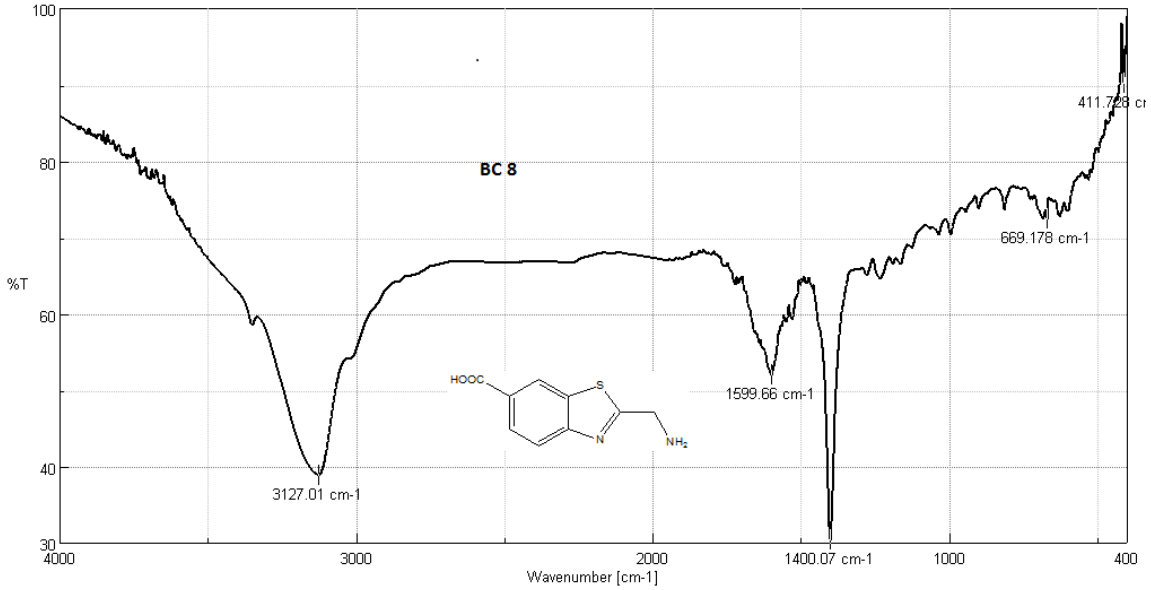


Fig 8: IR spectrum of compound BC 8

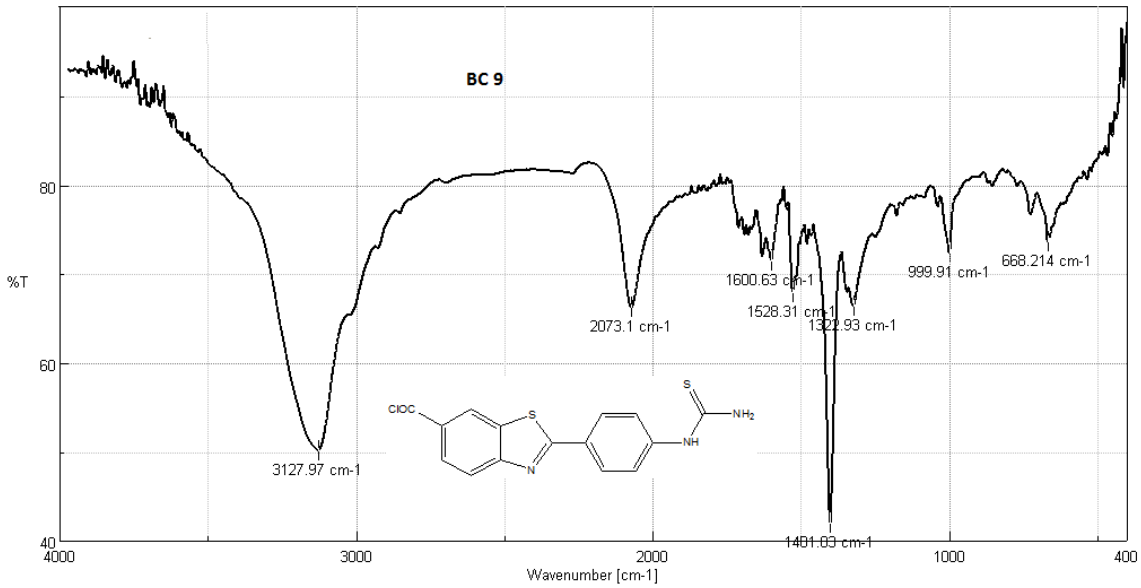


Fig 9: IR spectrum of compound BC 9

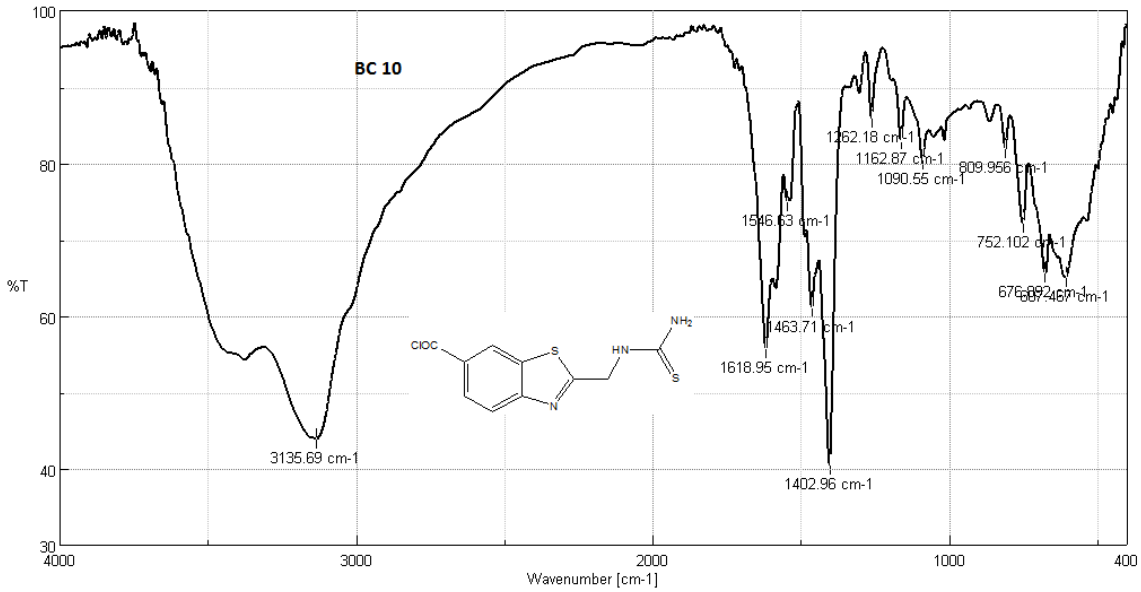


Fig 10: IR spectrum of compound BC 10

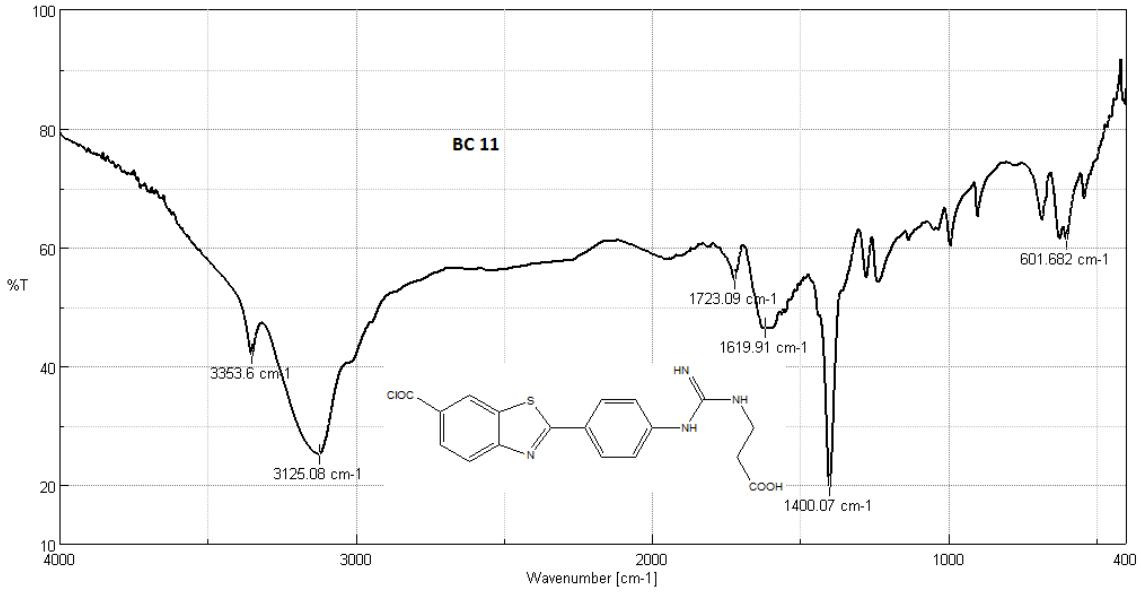


Fig 11: IR spectrum of compound BC 11

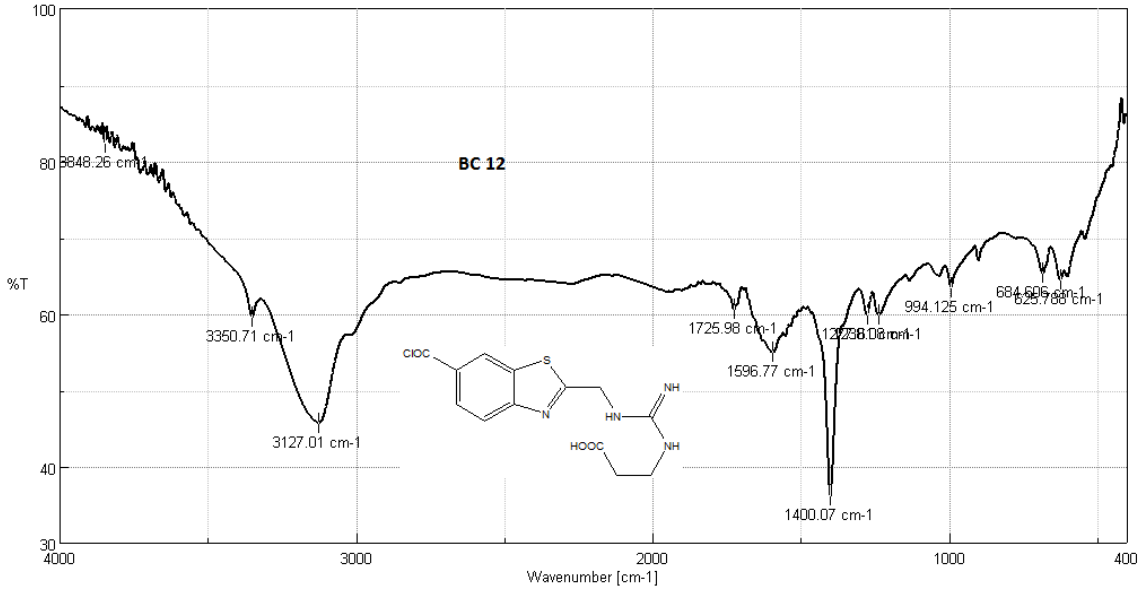


Fig 12: IR spectrum of compound BC 12

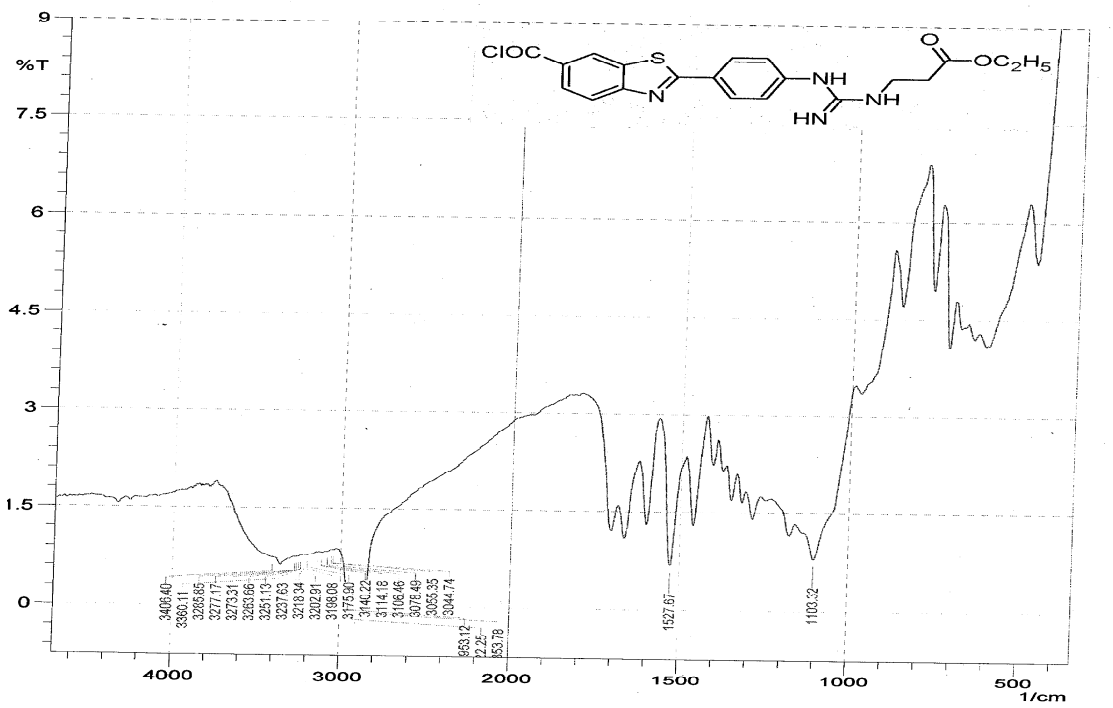


Fig 13: IR spectrum of compound BC 11a

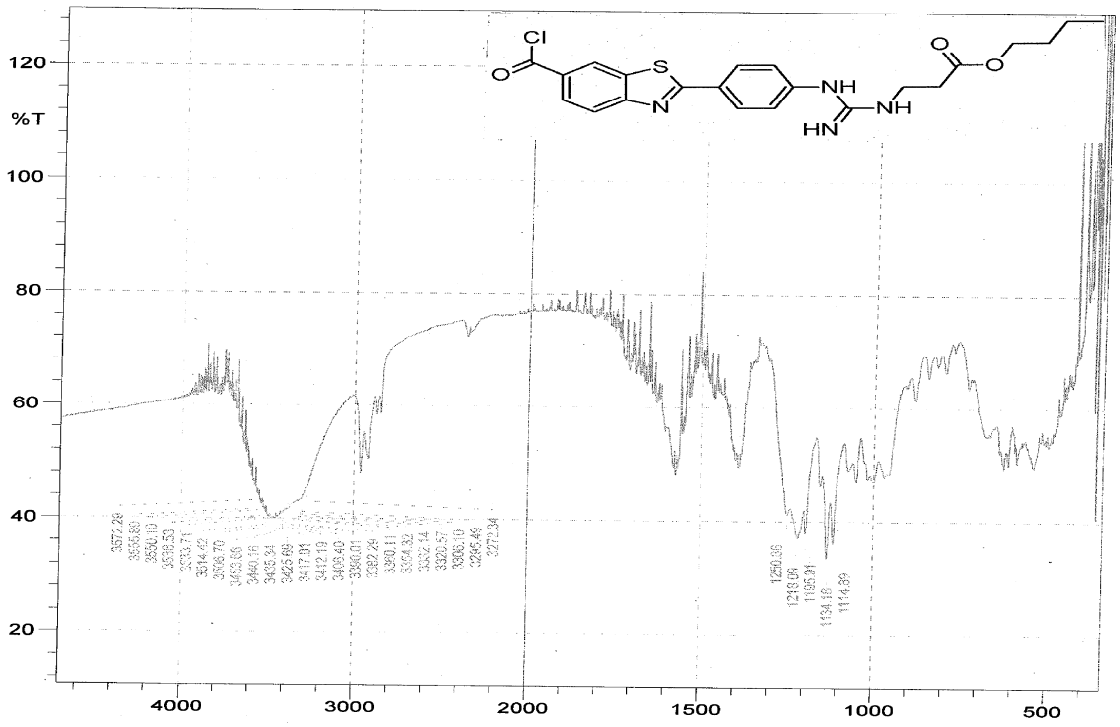


Fig 14: IR spectrum of compound BC 11b

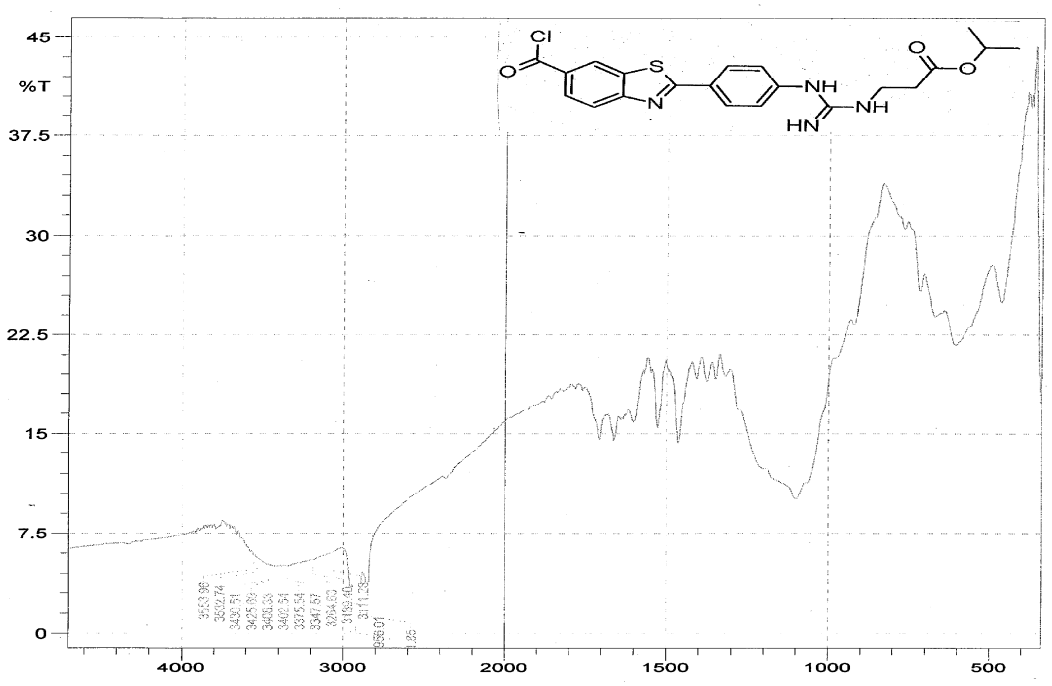


Fig 15: IR spectrum of compound BC 11c

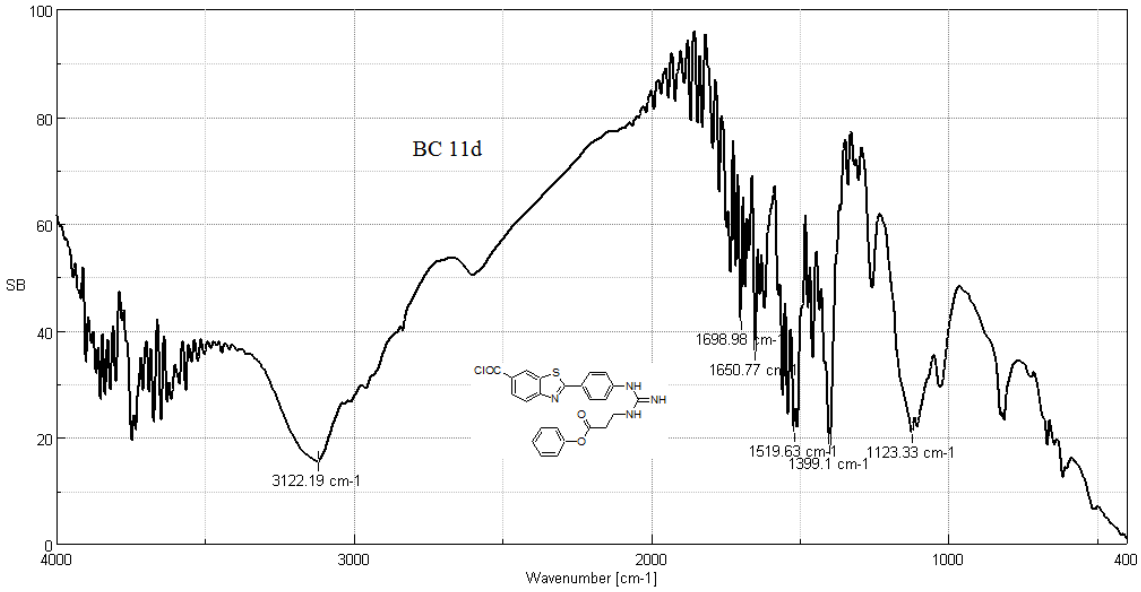


Fig 16: IR spectrum of compound BC 11d

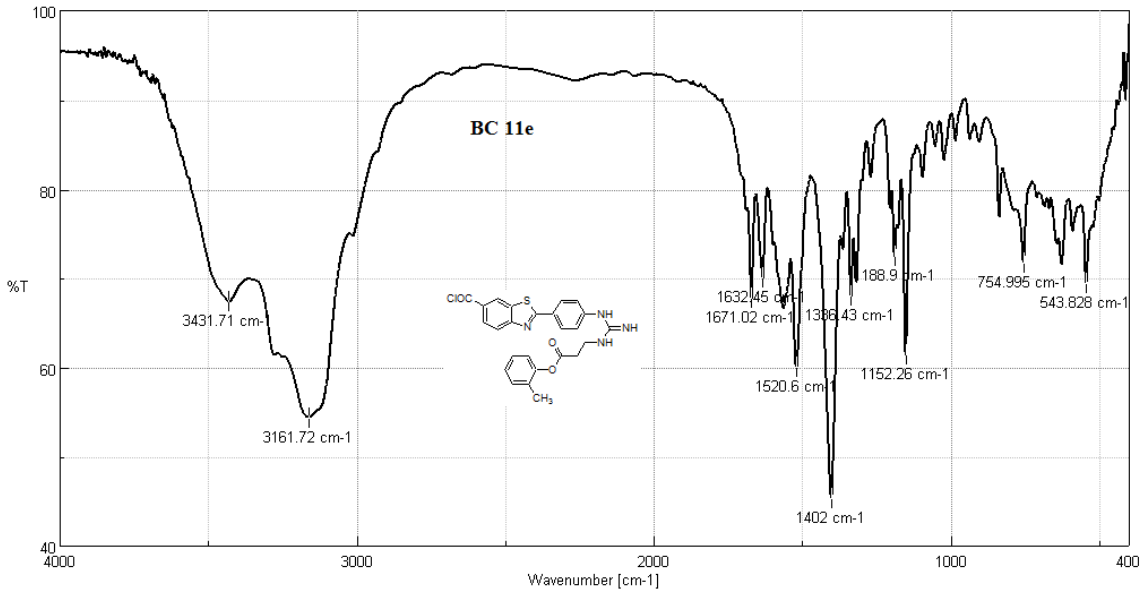


Fig 17: IR spectrum of compound BC 11e

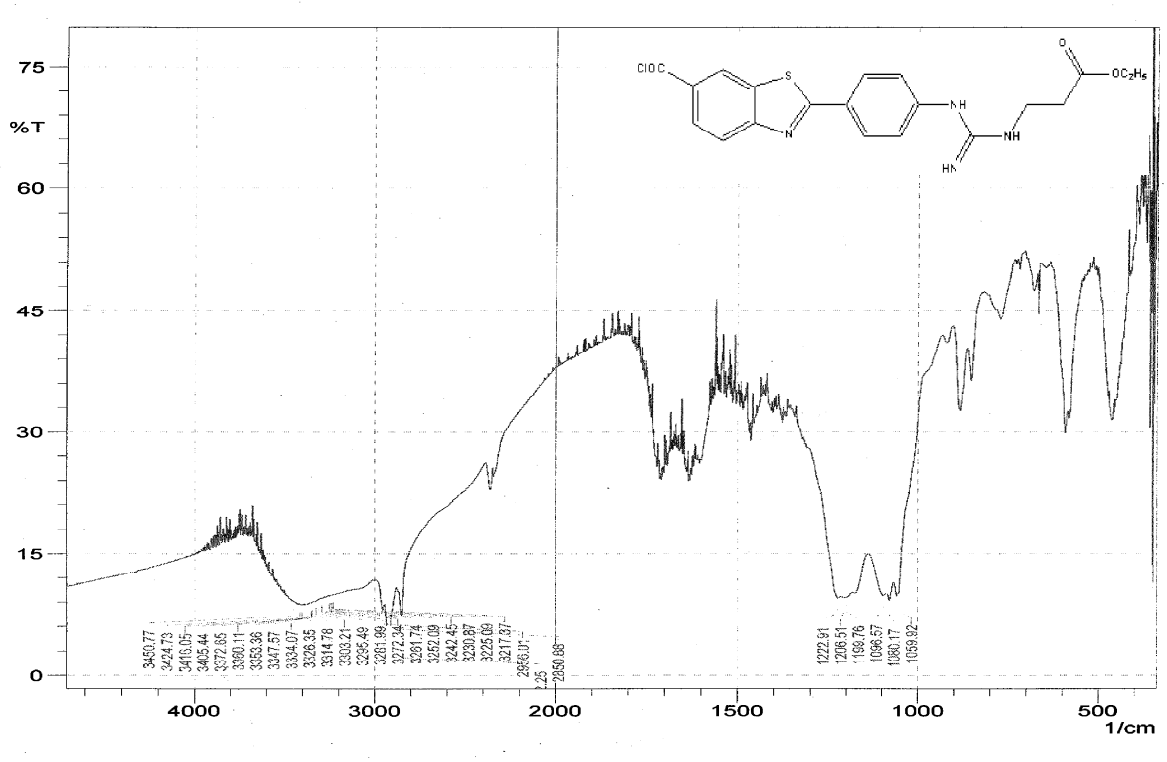


Fig 18: IR spectrum of compound BC 12a

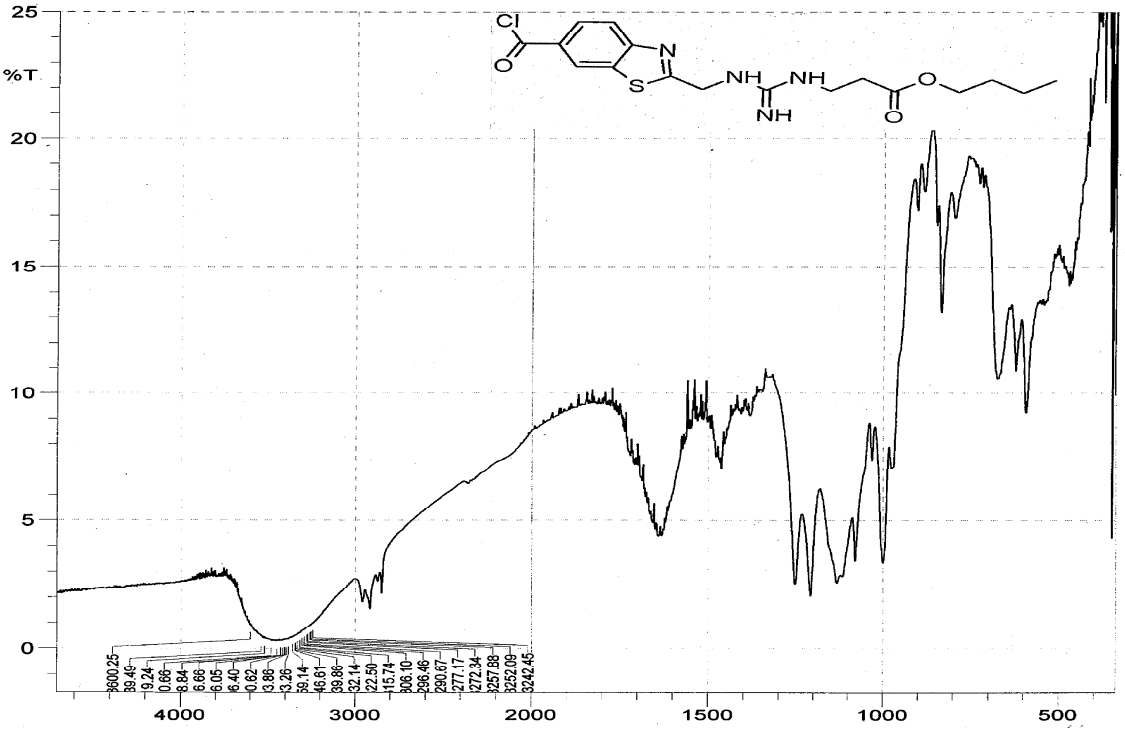


Fig 19: IR spectrum of compound BC 12b

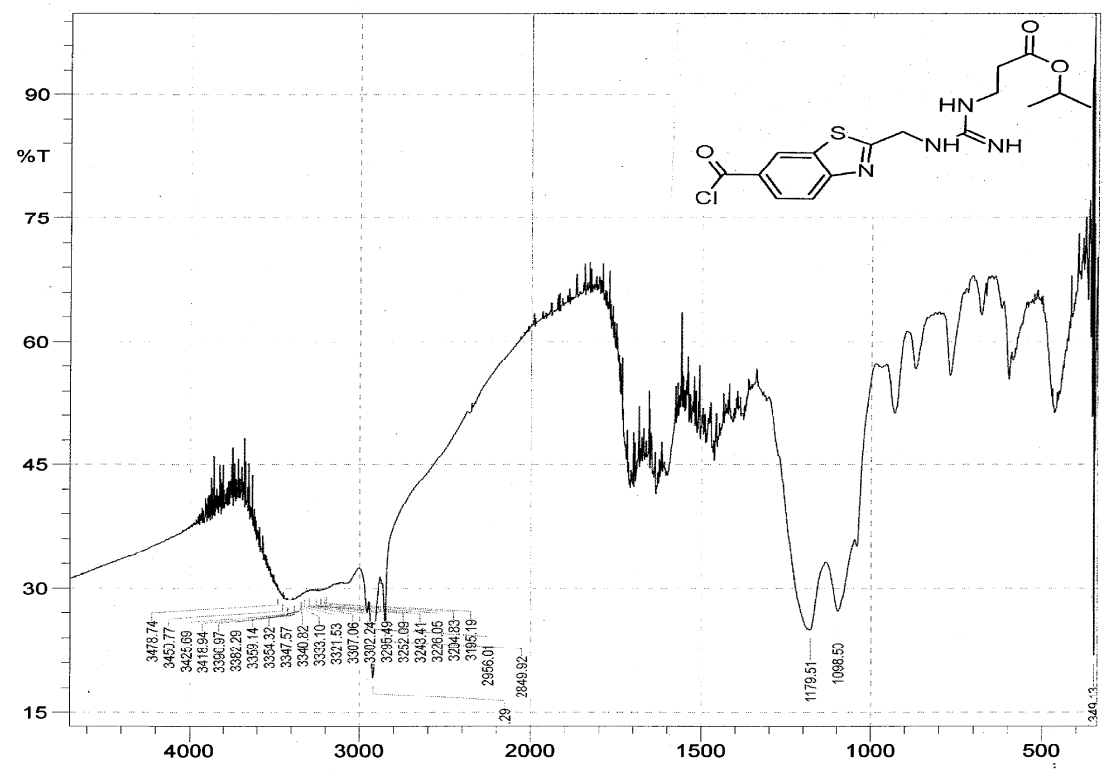


Fig 20: IR spectrum of compound BC 12c

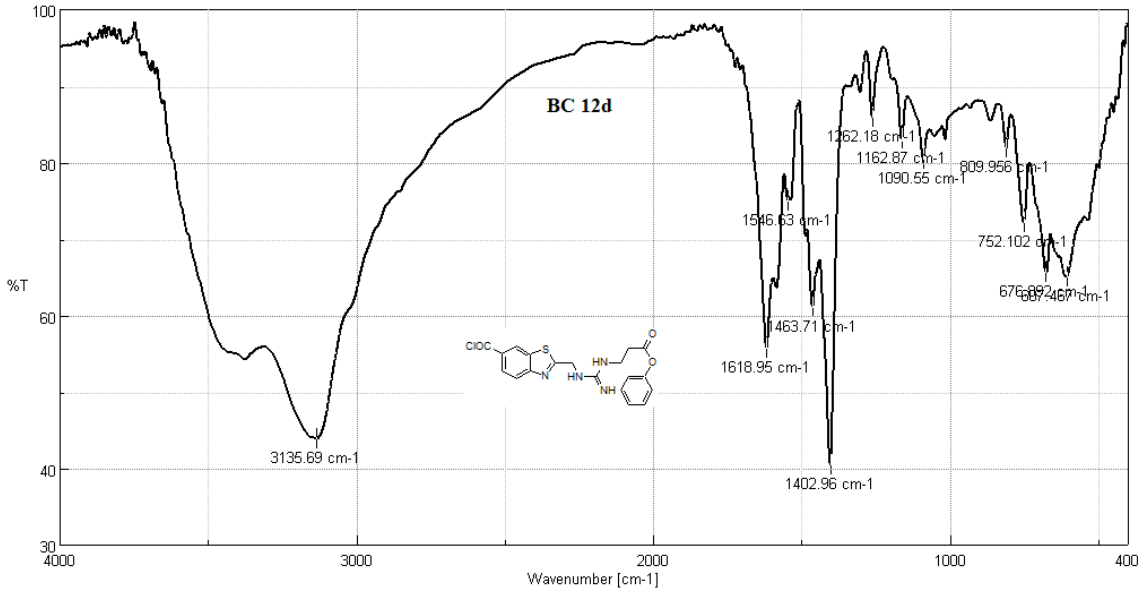


Fig 21: IR spectrum of compound BC 12d

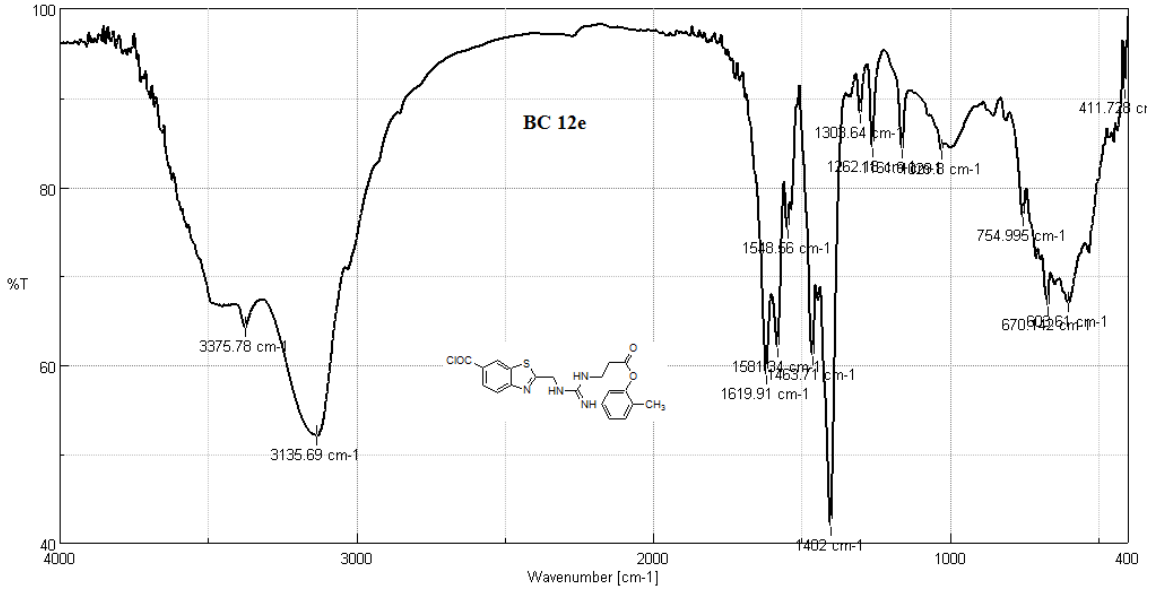


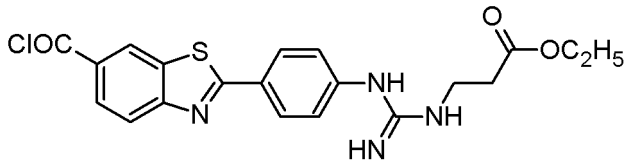
Fig 22: IR spectrum of compound BC 12e

3.5. NUCLEAR MAGNETIC RESONANCE SPECTROMETRY

Nuclear magnetic spectrometry is an important tool for determining the structures of a molecule. NMR spectrum can give almost unbelievable detailed information about molecular structure:

a. The number of signals: which tell us how many different kinds of protons there are in a molecule.
b. The positions of the signals, which tell us something about the electronic environment of each kind of proton.
c. The intensities of the signals, which tell us how many protons of each kind there are; and the splitting of a signal into several peaks, which tell us about the environment of a proton with respect to other, nearby protons. NMR spectral study was done on Burker Fourier, Transform-NMR Spectrometer on selected compounds

BC11a: ethyl-3-(N'-{4-[6-(chlorocarbonyl)-1, 3-benzothiazol-2-yl] phenyl} carbamimido) propanoate



8.39-8.20 (m, 3H, ArH in Benzothiazole)

7.95 (d, 4H, ArH in phenyl)

5.12 (s, 1H, NH)

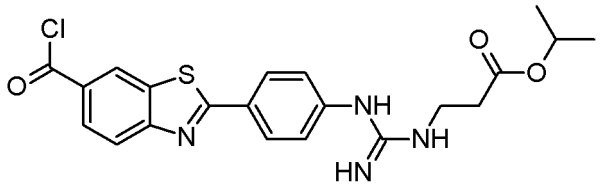
3.41 (s, 1H, NH)

2.50 (s, 2H, CH_2 in ester)

1.32-1.19 (t, 2H, CH_2)

0.85 (s, 3H, CH_3)

BC 11c: **isopropyl-3-(N'-{4-[6-(chlorocarbonyl)-1,3-benzothiazol-2-yl] phenyl} carbamimido)propanoate**



8.40-8.19 (m, 3H, ArH in Benzothiazole)

7.94-6.90 (d, 4H, ArH in phenyl)

4.30 (s, 1H, NH)

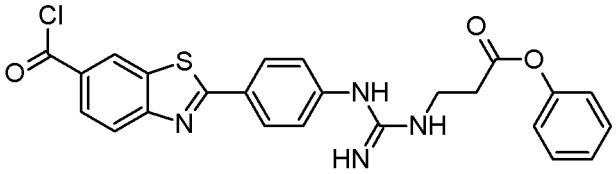
4.34-4.28 (m, 1H, CH)

3.70 (q, 2H, CH_2)

2.50-2.42 (t, 2H, CH_2)

1.34-1.09 (d, 6H, CH_3)

BC 11d: **phenyl-3-(N'-{4-[6-(chlorocarbonyl)-1, 3-benzothiazol-2-yl] phenyl} carbamimido) propanoate**



7.06-6.97 (m, 3H, ArH in Benzothiazole)

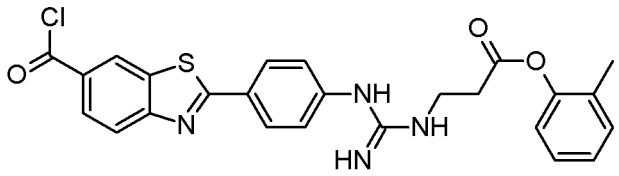
6.82-6.60 (d, 4H, ArH in phenyl)

6.70-6.67 (m, 5H, ArH in phenyl)

3.61 (s, 1H, NH)

2.14-2.16 (t, 2H, CH_2)

BC 11e: 2-methyl phenyl-3-(N'-{4-[6-(chlorocarbonyl)-1, 3-benzothiazol-2-yl] phenyl} carbamimido) propanoate



7.28-7.26 (m, 3H, ArH in Benzothiazole)

7.04-6.95 (d, 4H, ArH in phenyl)

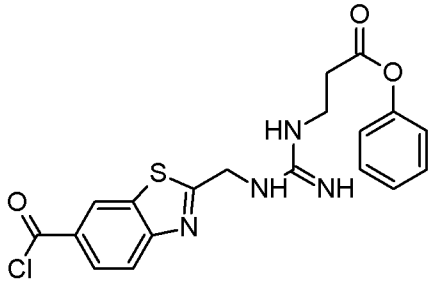
6.70-6.67 (m, 4H, CH in tolyl)

4.36 (s, 1H, NH)

2.51-2.50 (t, 2H, CH_2)

2.2-2.1 (s, 3H, CH_3)

BC 12d: phenyl-3-(N'-{4-[6-(chlorocarbonyl)-1, 3-benzothiazol-2-yl] methyl} carbamimido) propanoate

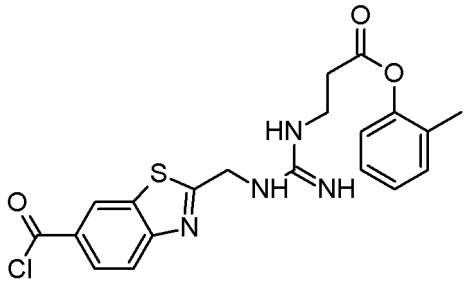


7.70-7.13 (m, 3H, ArH in Benzothiazole)

6.91-6.72 (m, 5H, ArH in phenyl)

2.60-2.50 (d, 2H, CH_2)

BC 12e: 2-methyl phenyl-3-(N'-{4-[6-(chlorocarbonyl)-1, 3-benzothiazol-2-yl] methyl} carbamimido) propanoate



7.17-7.14 (m, 3H, ArH in Benzothiazole)

6.77-6.74 (m, 4H, ArH in tolyl)

3.70-3.40 (d, 2H, CH_2)

2.50-2.30 (s, 3H, CH_3

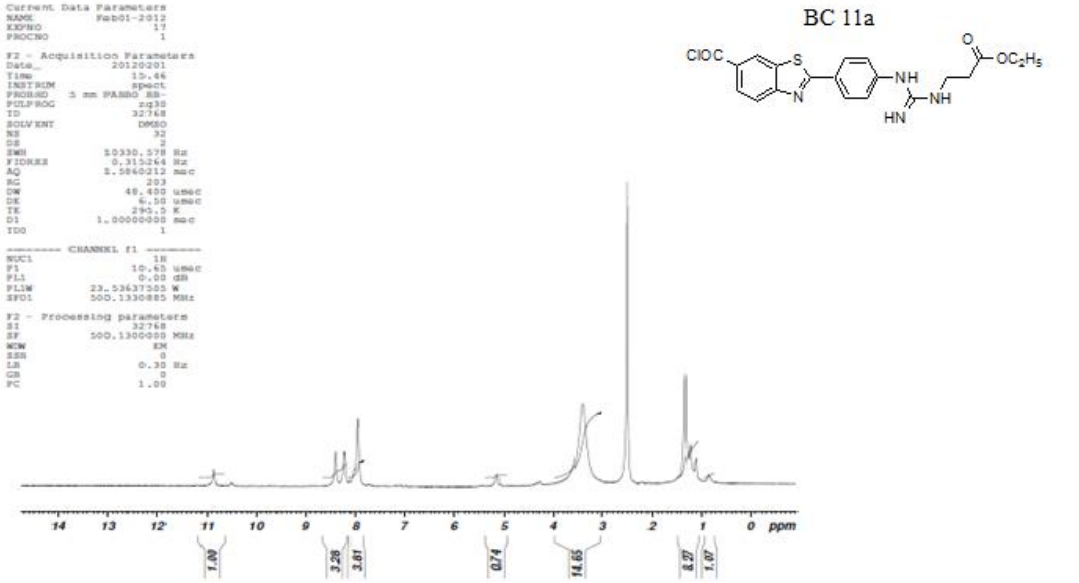


Fig 23: [1]HNMR spectrum of compound BC 11a

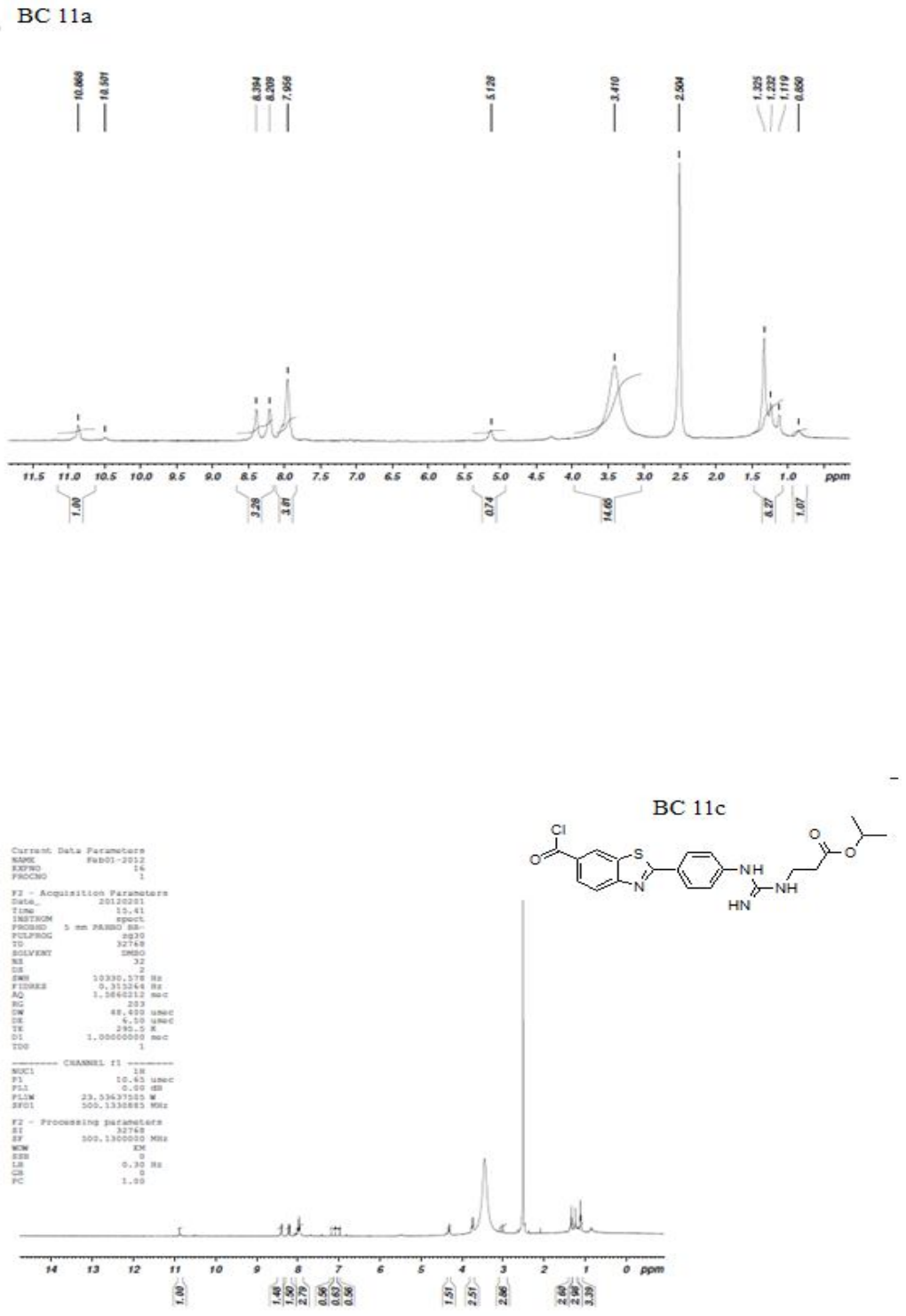
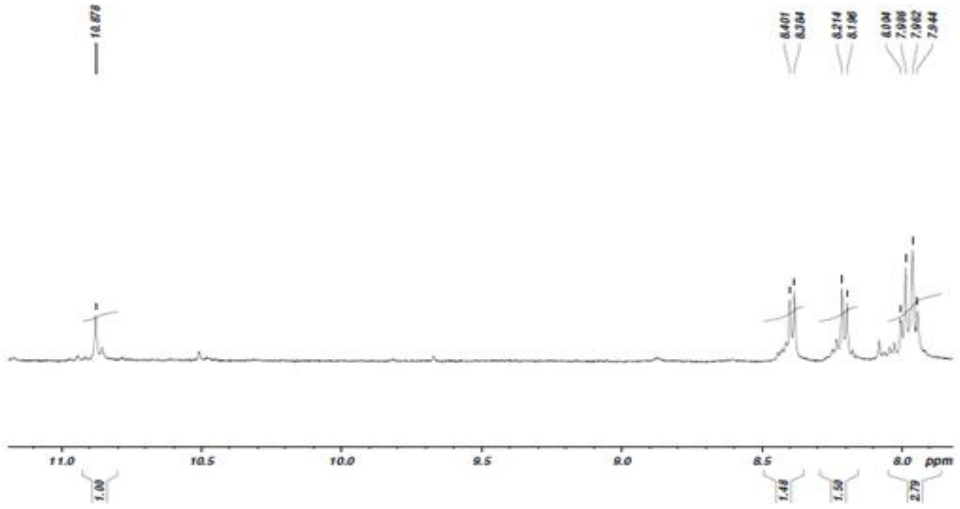
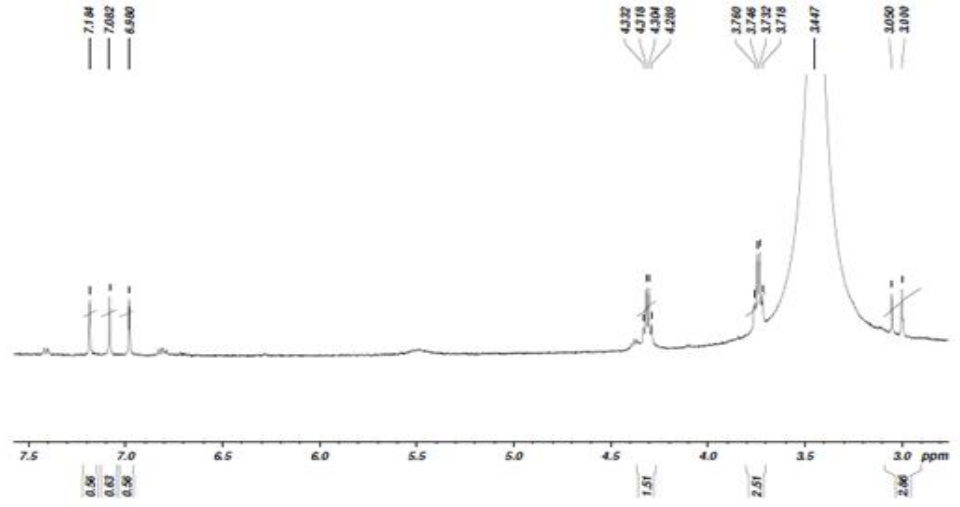


Fig 24: [1]HNMR spectrum of compound BC 11c

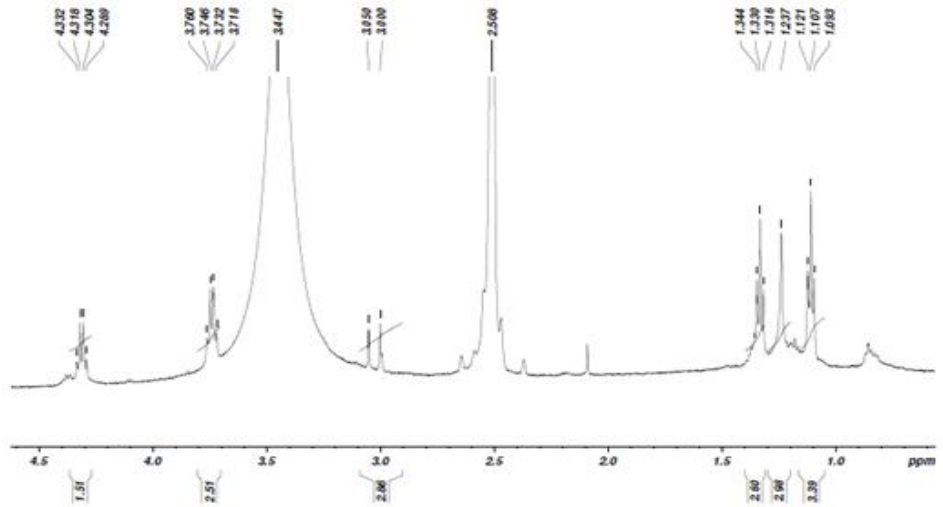
BC 11c


10.879
8.401
8.394
8.214
8.196
8.004
7.988
7.962
7.944
11.0
10.5
10.0
9.5
9.0
8.5
8.0
ppm
1.00
1.48
1.50
2.79

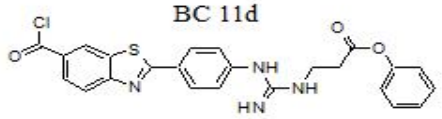
BC 11c


7.184
7.082
6.980
4.332
4.318
4.304
4.289
3.760
3.746
3.732
3.718
3.447
3.050
3.036
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
ppm
0.56
0.63
0.56
1.51
2.51
2.66

BC 11c



BC 11d



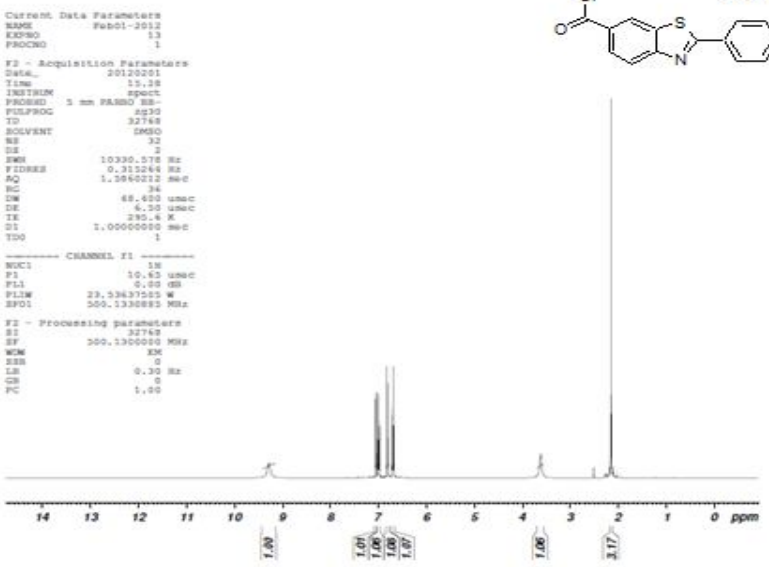
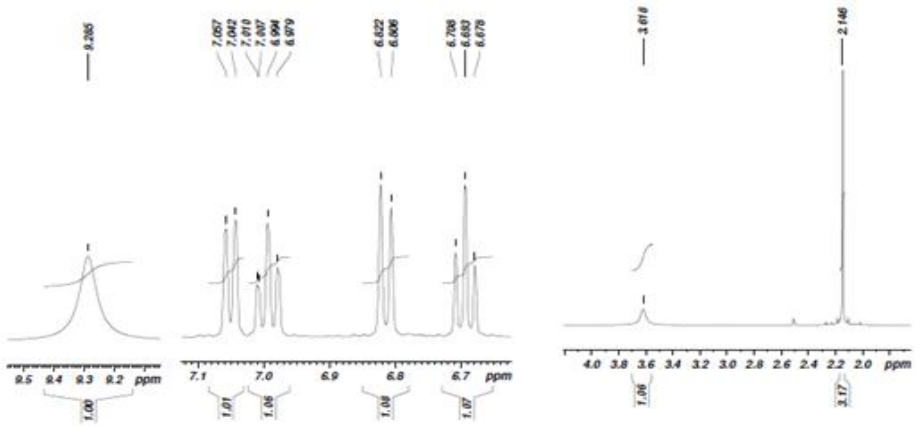
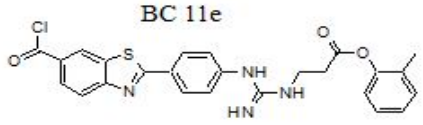


Fig 25: [1]HNMR spectrum of compound BC 11d

BC 11d



BC 11e



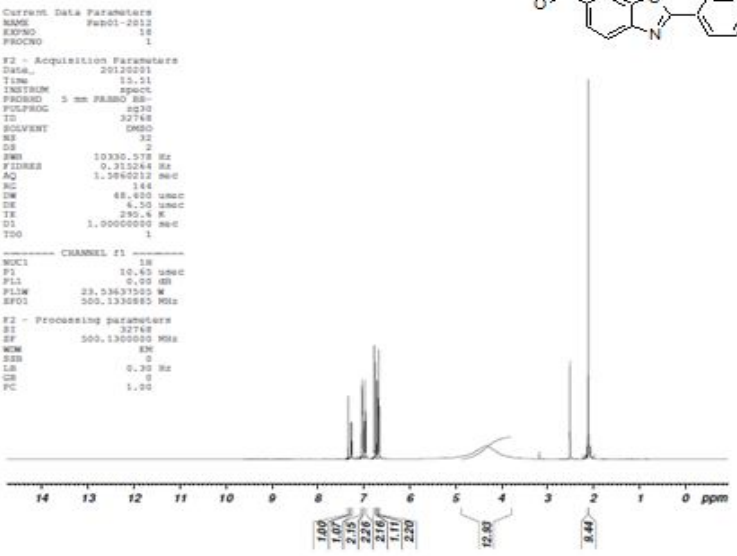
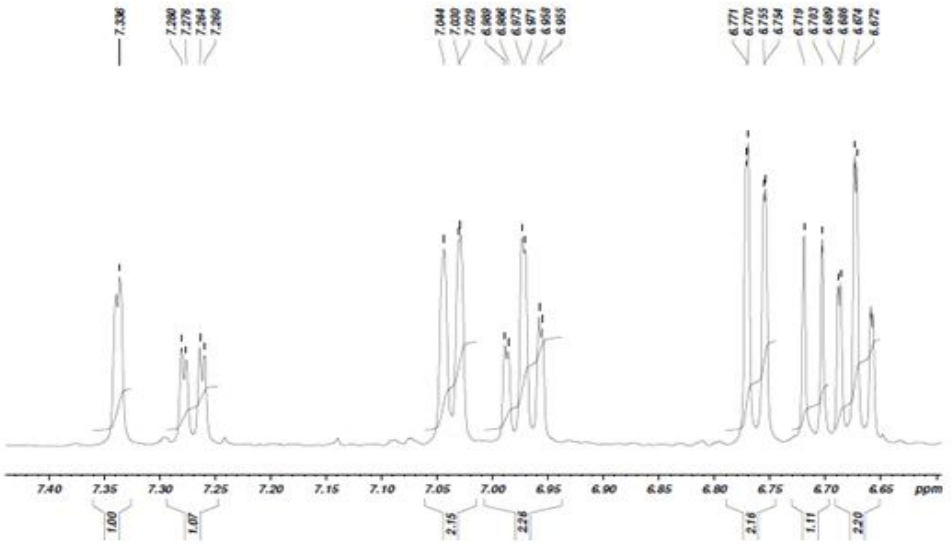
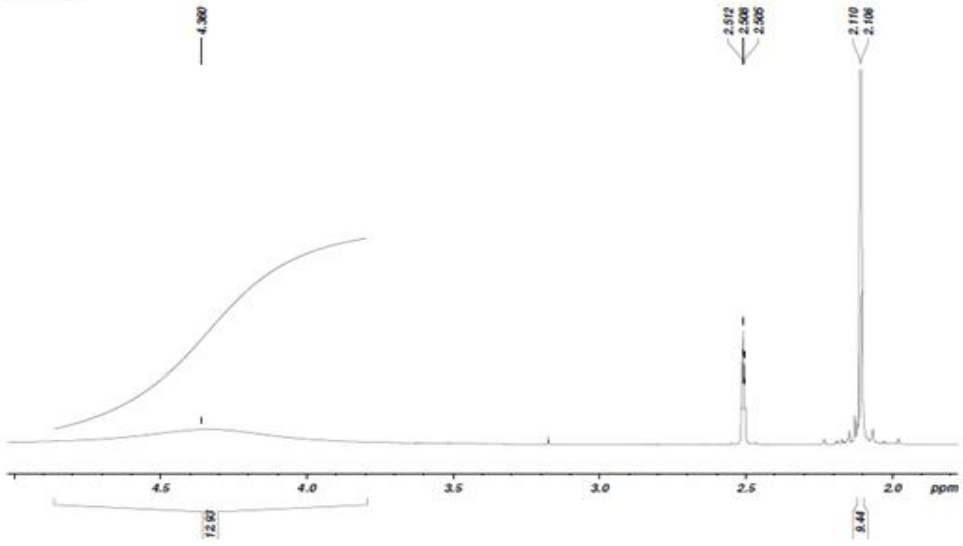


Fig 26: [1]HNMR spectrum of compound BC 11e

BC 11e


7.336
7.280
7.278
7.264
7.260
7.044
7.030
7.029
6.989
6.986
6.973
6.971
6.958
6.955
6.771
6.770
6.755
6.754
6.719
6.703
6.689
6.686
6.674
6.672
7.40
7.35
7.30
7.25
7.20
7.15
7.10
7.05
7.00
6.95
6.90
6.85
6.80
6.75
6.70
6.65
ppm
1.00
1.07
2.15
2.26
2.16
1.11
2.20

BC 11e


4.300
2.512
2.508
2.505
2.110
2.106
4.5
4.0
3.5
3.0
2.5
2.0
ppm
12.93
9.44

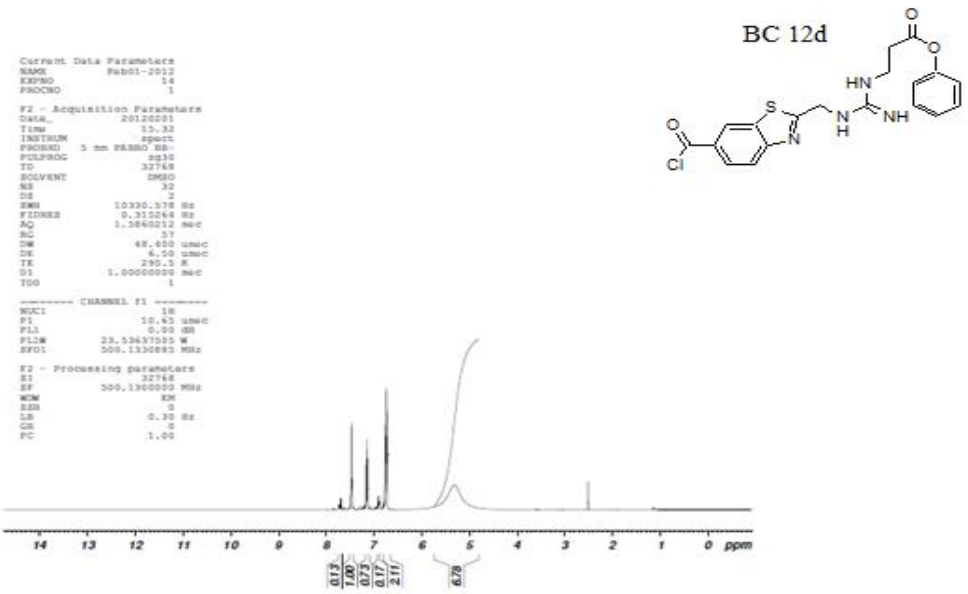
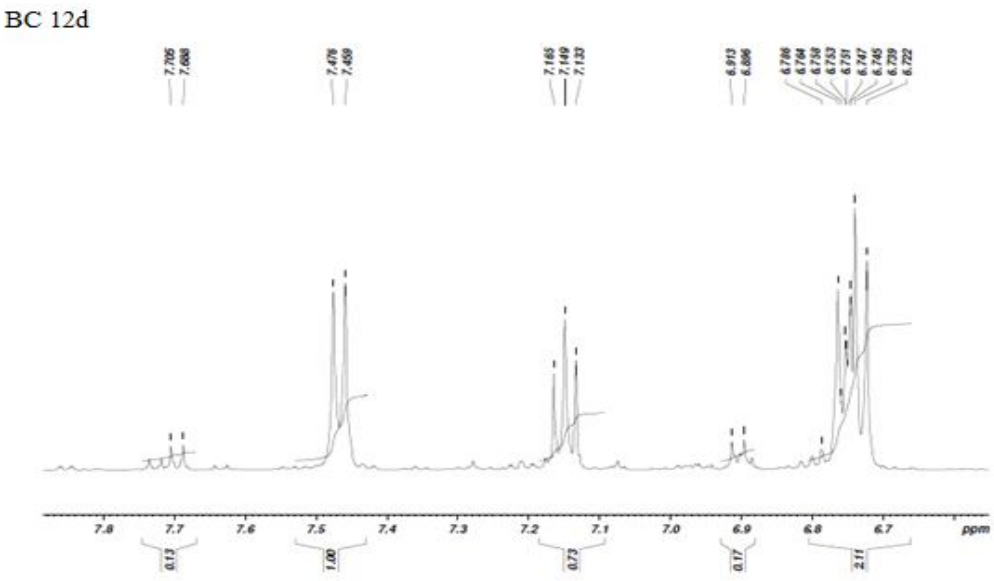


Fig 27: [1]HNMR spectrum of compound BC 12d



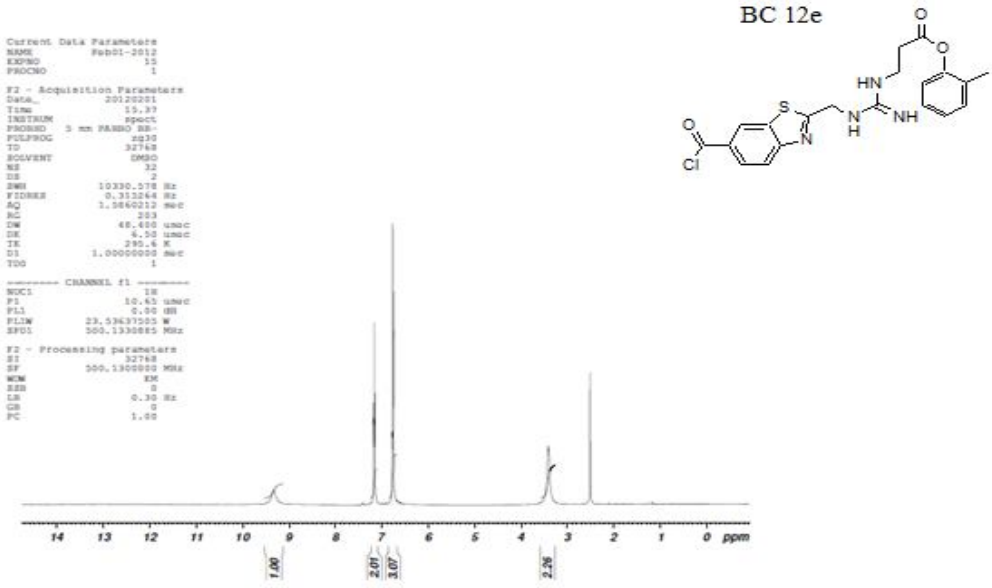
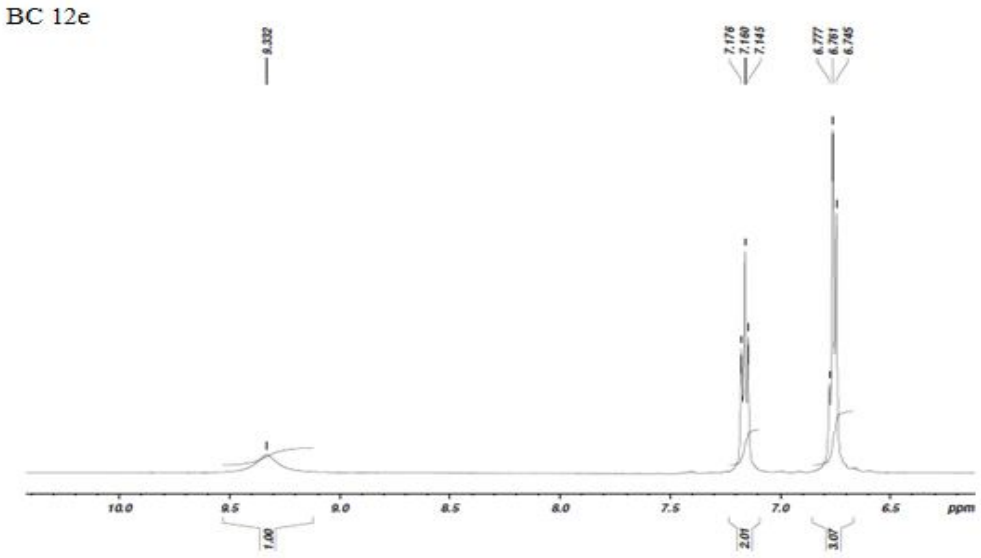


Fig 28: [1]HNMR spectrum of compound BC 12e



3.6. MASS SPECTROSCOPY

In the technique of mass spectroscopy, the compound under investigation is bombarded with a beam of electrons which produce an ionic molecule or ionic fragments of the original species. The resulting assortment of charged particles is then separated according to their masses. The spectrum produced, known as mass spectrum is a record of information regarding various masses produced and their relative abundance.

The mass spectra of selective compounds were recorded on JEOL GCMATE instrument.

Table 4

S.NO	Compound code	Molecular mass(m\z)	Base peak	[M+2] peak
1	BC 11d	478.60	251.39	480.02
2	BC 12e	430.34	251.34	432.01

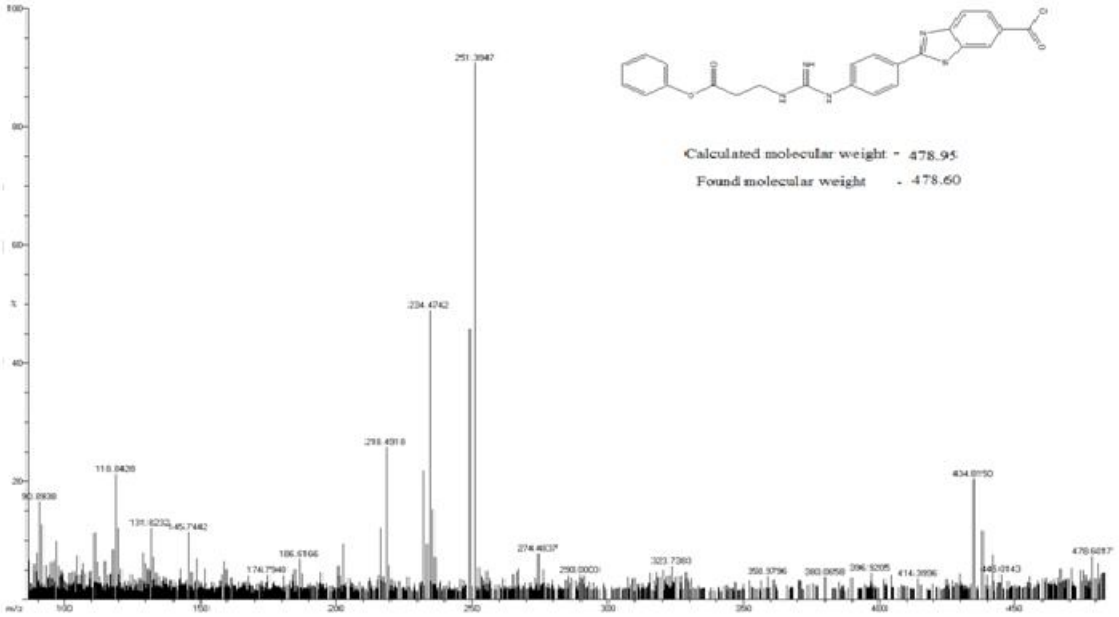


Fig 29: Mass spectrum of compound BC 11d

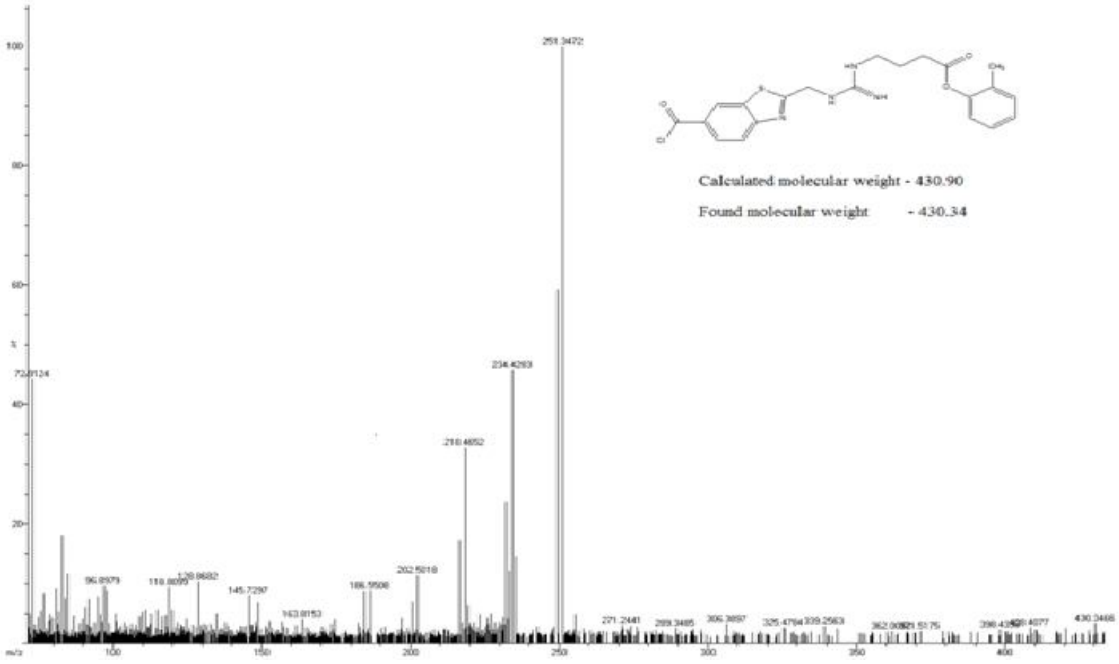


Fig 30: Mass spectrum of compound BC 12e

3.7. *IN-VITRO* ALDOSE REDUCTASE ENZYME INHIBITON ASSAY TECHNIQUE[13, 14]

Procedure:

The Aldose reductase enzyme was purchased from the Sigma-Aldrich. The AR activity was spectrophotometrically assayed by measuring the decrease in NADPH absorption at 340 nm over a 4 min period, using DL-glyceraldehyde as a substrate. Each 1.0 ml cuvette containing equal units of enzyme, 0.1M sodium phosphate buffer (pH 6.2), 0.3 mM NADPH either with or without 10 mM substrate and inhibitor (10µg) was prepared. One set of mixtures prepared with an equivalent volume of sodium phosphate buffer instead of tested samples was used as control. The concentration of the sample required to inhibit 50% of AR activity under the assay conditions was defined as the IC_{50} value. Epalrestat was used as a standard drug. Inhibitory concentration (IC_{50}) was calculated by using the following formula.

$$\mathbf{IC_{50} = (1\text{-}A|B) \times 100}$$

Where, A= Absorbance of sample

B= Absorbance of control

Table 5: *In vitro* Aldose reductase inhibitory activity data of synthesized compounds

S.N	Compounds	Absorbance	Ic_{50} (%)
1	BC 11a	1.4872	26
2	BC 11b	1.5812	21
3	BC 11c	1.4015	30
4	BC 11d	0.6532	59
5	BC 11e	0.7563	53
6	BC 12a	1.4225	29
7	BC 12b	1.5581	22
8	BC 12c	1.5779	31
9	BC 12d	0.3235	80
10	BC 12e	0.5063	68
11	Epalrestat	0.2012	90

3.8. DOCKING STUDIES WITH ALDOSE REDUCTASE[15]

Procedure:

Molecular Operating Environment (MOE) 2007[16] was used for ligand and protein preparation and molecular structure viewing. The crystal structure of Aldose reductase was obtained from the Protein Data Bank with the accession code 2BGQ. Docking calculations were conducted with AUTODOCK 4.1 In short; AUTODOCK performs an automated docking of the ligand with user-specified dihedral flexibility within a protein rigid binding site. The program performs several runs in each docking experiment. Each run provide some predicted binding mode. All water molecules, zinc atom, ligand were removed from the Protein Data Bankfile. Polar hydrogen atoms were added and Kollman charges, atomic salvation parameters and fragmental volumes were assigned to the protein. For validation of the docking protocol, ligand coordinates in the crystal complex were removed. For ligand (BC 12d), gasteiger charges were assigned and non-polar hydrogen atoms were merged. All torsions were allowed to rotate during docking. The auxiliary program Auto Grid generated the grid maps. Lennard-Jones parameters 12–10 and 12–6, supplied with the program, were used for modeling H-bonds and van der Waals interactions, respectively. The distance-dependent dielectric permittivity of Mehler and Solmajer was used for calculation of the electrostatic grid maps. For ligand, random starting positions, random orientations and torsions were used. The translation, quaternion and torsions steps were taken from default values in AUTODOCK. The Lamarckian genetic algorithm and the pseudo-Solis and Wets methods were applied for minimization using default parameters. The number of docking runs was 10. After docking, the 10 solutions were clustered into groups with RMS lower than 1.0 Å. The clusters were ranked by the lowest energy representative of each cluster. In order to describe the ligand-binding pocket interactions, the top ranked binding mode found by AUTODOCK in complex with the binding pocket of Aldose reductase was subject to full energy minimization using the MMFF94 force field implemented in MOE until the gradient 0.05 was reached. During minimization, residue atoms within 8 Å from the ligand were free to move (other atoms were fixed).

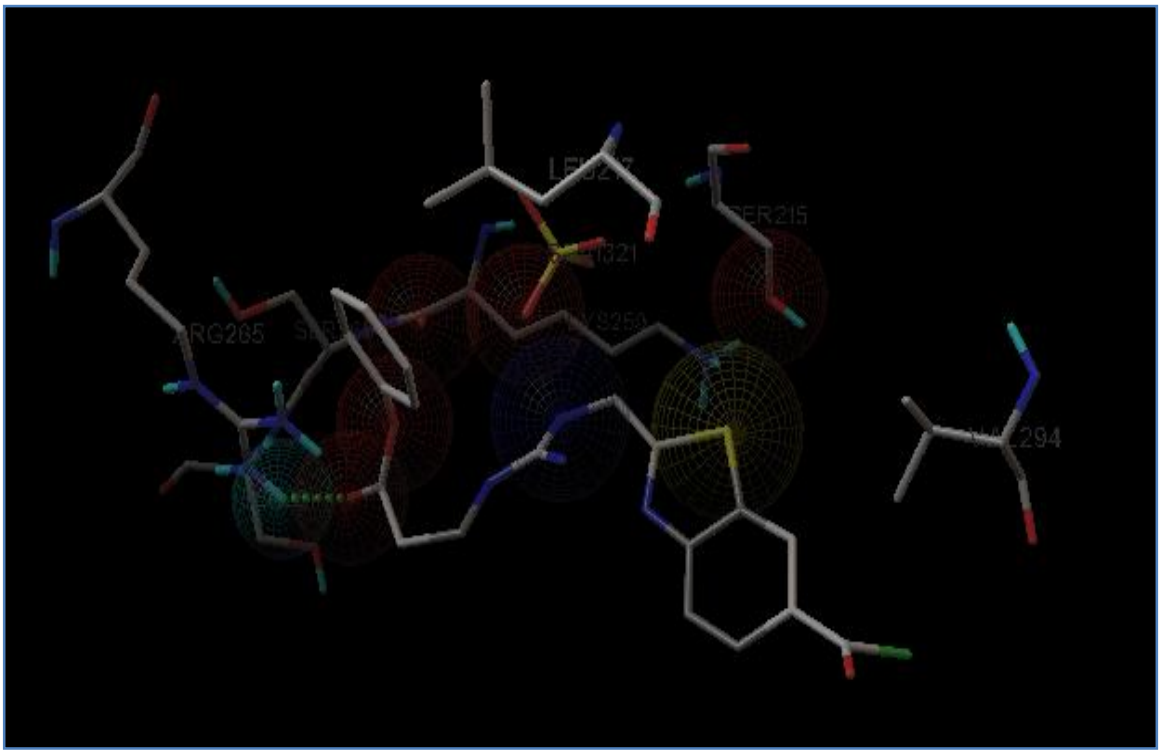


Fig 31: Predicted binding mode of BC 12d into the catalytic site of AR. Amino acid residues to the binding pocket of ligand (2.25 Å) are labeled

3.9. *IN-VITRO* ANTIMICROBIAL STUDIES[17]

The *in vitro* antibacterial activity of the series of synthesized compounds was evaluated against 9 pathogenic bacteria and 4 pathogenic fungi. The bacteria and fungus strains were procured from the bacterial repository of department of biotechnology, KMCH College of Pharmacy, Coimbatore.

Dimethyl sulphoxide was used to prepare stock solution of standard and synthesized drugs. Subsequent dilutions of the stock were also made with above solvent. Muller Hinton agar media (hi-media) for bacteria and Sabourand's Dextrose Broth for fungus were used to subculture various strain of bacteria and fungus as well for determining the MIC's of synthesized compounds by serial dilution method.

3.9.1. DISC DIFFUSION METHOD

The disc diffusion method, the drug potency is based on measurement of the diameter of zone of inhibition surrounding cylinder disc which are placed on the surface of a nutrient medium previously inoculated with a culture of suitable microorganisms. Inhibition produced by the test compound is compared with that produced by known concentration of reference standard ascorbic acid.

List of bacterial and antifungal strains used

Bacteria

1. *Micrococcus luteus (*Gram positive)
2. *Staphylococcus aureus (*Gram positive*)*
3. *Corny bacterium diphtheria (*Gram positive*)*
4. *Bacillus subtilis (*Gram positive*)*
5. *Bacillus lintus (*Gram negative*)*
6. *Escherichia coli (*Gram negative*)*
7. *Pseudomonas aeruginosa (*Gram negative*)*
8. *Rhodosporium rubrum (*Gram negative*)*
9. *Vibrio cholera (*Gram negative*)*

Fungus

1. *Candida albicans*
2. *Aspergillus niger*
3. *Aspergillus fumigates*
4. *Aspergillus parasites*

3.9.1. a. Antibacterial screening

The antibacterial activities of synthesized compounds were screened in the concentration of 0.1mg in dimethyl sulphoxide against the above mentioned bacteria in the Muller Hinton agar medium by Disc diffusion method using ciprofloxacin as standard. The antibacterial activity was evaluated by measuring zone of inhibition in mm. the procedure is given below.

PROCEDURE

Preparation of Nutrient Broth for Bacteria

Ingredients used

S.N	Ingredients	Quantity
1	Beef extract	10g
2	Peptone	10g
3	Sodium chloride	5g
4	Water	1000ml

The accurately weighed quantity of above ingredients were transferred to a conical flask, and dissolved in distilled water with the aid of heat with stirring and the pH was adjusted to 7.2 – 7.4 and plugged with non-absorbent cotton, covered by Aluminum foil and sterilized by autoclaving (121^0C at 15 Ibs pressure for 15 min).

Preparation and standardization of inoculation

Each bacterial pure culture from the slant culture is picked up aseptically and was transferred into 100 ml of nutrient broth. The inoculated broths were incubated at 37^0C for 24 hrs and growth was arrested by stored in the refrigerator (below 4^0C).

Sample Preparation

0.9mg of each sample was dissolved in 1ml DMSO (Dimethyl sulphoxide). The solvent control shows no antibacterial activity with all the test organisms used. The sterile disc (6 mm in diameter) were impregnated with 100 µg/disc of the sample and tested against microbial cultures.

Media used (Muller-Hinton Agar Medium, Hi-media India (Pvt) Ltd)

Muller Hinton agar medium was prepared and transferred into sterile Petri plates aseptically (thickness of 5-6mm). The plates were allowed to dry at room temp. The plates were inverted to prevent condensate falling on the agar surface. The layers of the medium are uniform in thickness, is done by placing the plates on a leveled surface. Standardized bacterial inoculums were applied to the plates and spreaded uniformly over the surface of medium by using a sterile Non-absorbent cotton swab and finally the swab was passed around the edge of the medium. The inoculated plates were closed with the lid and allowed to dry at room temperature. The sample impregnated discs were placed on the inoculated agar medium. All Petri plates were incubated at 37^0C for 24 hrs. After the incubation diameter of zone of inhibition produced by the sample were measured. (Table-6, Fig-(32 to 34))

3.9.1. b. Antifungal screening

The antifungal activities of synthesized compounds were screened in the concentration of 0.1mg in dimethyl sulphoxide against the above mentioned fungus in the Sabourand's Dextrose Agar medium by Disc diffusion method using Clotrimazole as standard. The antifungal activity was evaluated by measuring zone of inhibition in mm. The procedure is given below.

PRCEDURE

Preparation of Sabourand's Dextrose Broth for Fungi

Ingredients used

S.N	Ingredients	Quantity
1	Dextrose	40g
2	Peptone	10g
3	Water	1000ml

Specified amount of peptone and dextrose was taken along with 1000ml of distilled water in a conical flask and heated in a steam bath to dissolve. The pH was maintained at 7.6 ± 0.2 and sterilized in an autoclave at 15 lb pressure, 120^0C for 15 minutes. The sterile medium was poured into the Petri dish and allowed to solidify.

Preparation and standardization of inoculums

Each fungal pure culture from the slant culture is picked up aseptically and was transferred into 100ml of nutrient broth. The inoculated broths were incubated at 27^0C-28^0C for 24-48 hrs and growth was arrested by stored in the refrigerator (below 4^0C).

Sample preparation

0.4mg of each sample was dissolved in 0.5ml DMSO (Dimethyl Sulphoxide). The solvent control shown no antibacterial activity with all the test organisms used. The sterile disc (6 mm in diameter) were impregnated with 100µg/disc of the sample and tested against microbial cultures.

Media Used

Sabourand's Dextrose Agar medium, Hi-media India (Pvt) Ltd

Sabourand's dextrose Agar medium was prepared and transferred into sterile petriplates aseptically (thickness of 5-6mm). The plates were allowed to dry at room temp. The plates were inverted to prevent condensate falling on the agar surface. The layers of the medium are uniform in thickness, is done by placing the plates on a leveled surface. Standardized bacterial inoculums were applied to the plates and spreaded uniformly over the surface of medium by using a sterile Non-absorbent cotton swab and finally the swab was passed around the edge of the medium. The inoculated plates were closed with the lid and allowed to dry at room temperature. The sample impregnated discs were placed on the inoculated agar medium. All Petri plates were incubated at 27^0C-28^0C for 24 hrs. After the incubation diameter of zone of inhibition produced by the sample were measured. (Table-8, Fig-35)

3.8.2. MINIMUM INHIBITORY CONCENTRATION (MIC)

The lowest concentration of the test compound that completely inhibited growth on agar plates, disregarding a singly colony or a faint haze caused by the inoculums was considered as minimum inhibitory concentration (MIC) of that compound.

The potency of drug is based on inhibition of microbial growth as indicated by measurement of turbidity of a suspension of a suitable microorganism in a fluid medium to which graded amount of test compound have been added. The changes in turbidity produced by known concentration of reference materials are compared with results.

Determination of Minimum Inhibitory Concentration for Synthesized Compounds (MIC) by serial dilution Method:

1. The serial dilutions of known concentration of compound solution were made from the stock (0.1 mg/ml) by using Muller Hinton broth using the method described below.

2. The tubes were labeled 1 to 8 and 1 ml of Muller Hinton broth were added to the first 5 tubes and 8^{th} tube, then added 0.5 ml Muller Hinton broth to 6^{th} and 7^{th} tubes.
3. One ml of different synthesized compounds was added to the 1^{st} tube, mixed and transfers 1 ml serially up to tube 5. From the 5^{th} tube transfer 1ml to 6^{th} tube. Mixed and transfer 0.5 ml to the 7^{th} tube. Each tube, 1 to 7 contains 1ml diluted extract.
4. 8^{th} tube was the control.
5. With a standardized micro pipette, added a drop of the diluted broth culture approximately 0.01ml of the test organism to all tubes, including the control, gently mixed and incubated at $37\ ^{0}C$ for 16 to 18hrs.
6. The highest dilution of particular compounds showing no turbidity was observed and recorded. This was taken as the end point, and this dilution was considered to contain the concentration of drug equivalent to MIC. (Table-7, Table-9).

3.9.3. RESULT

ANTI-BACTERIAL SCREENING [DISC DIFFUSION METHOD]

Table 6: Anti-bacterial activity of the synthesized compounds

S.N	Micro organism	Zone of Inhibition (in mm)										
		BC11a	**BC11b**	**BC11c**	**BC11d**	**BC11e**	**BC12a**	**BC12b**	**BC12c**	**BC12d**	**BC12e**	**STD**
1	*Micrococcus luteus*	11	12	11	14	15	13	13	12	15	14	15
2	*Bacillus linctus*	12	10	11	15	14	10	11	9	14	15	17
3	*Staphylococcus aureus*	11	11	12	15	13	9	9	10	13	13	16
4	*Bacillus subtilis*	12	13	13	14	14	11	8	10	13	14	15
5	*Salmonella paratyphi*	10	11	13	15	14	11	9	9	15	14	15
6	*Pseudomonas aureginosa*	12	12	12	14	15	10	8	9	16	15	18
7	*Klebseilla pneumonia*	11	10	13	14	15	13	12	10	14	15	15
8	*Vibrio cholera*	9	12	12	13	14	12	11	11	13	13	16
9	*Escherichia coli*	12	11	11	15	13	12	12	10	13	14	15

STD: ciprofloxacin (5µg\disc)

SERIAL DILUTION METHOD

Table 7: MIC values of the synthesized compounds

S.N	Micro organism	MIC values (µg/ml)										
		BC11a	**BC11b**	**BC11c**	**BC 11d**	**BC11e**	**BC12a**	**BC12b**	**BC12c**	**BC12d**	**BC12e**	**STD**
1	*Micrococcus luteus*	50	25	25	12.5	12.5	25	25	25	12.5	12.5	1.25
2	*Bacillus linctus*	25	50	25	12.5	12.5	25	25	50	12.5	12.5	3.25
3	*Staphylococcus aureus*	25	25	12.5	12.5	12.5	50	50	25	12.5	12.5	1.56
4	*Bacillus subtilis*	25	12	12.5	12.5	12.5	25	50	50	12.5	12.5	2.01
5	*Salmonella paratyphi*	50	25	12.5	12.5	12.5	25	50	50	12.5	12.5	1.08
6	*Pseudomonas aureginosa*	25	25	25	12.5	12.5	50	50	50	12.5	12.5	1.03
7	*Klebseilla pneumonia*	50	50	12.5	12.5	12.5	125	25	50	12.5	12.5	1.56
8	*Vibrio cholera*	50	25	25	12.5	12.5	25	25	25	12.5	12.5	1.56
9	*Escherichia coli*	25	25	25	12.5	12.5	25	25	50	6.25	6.25	1.06

STD: ciprofloxacin (100µg\ml)

ANTI-FUNGAL SCREENING [DISC DIFFUSION METHOD]

Table 8: Anti-fungal activity of the synthesized compounds

S.N	Micro organism	Zone of inhibition (in mm)										
		BC11a	**BC11b**	**BC11c**	**BC11d**	**BC11e**	**BC12a**	**BC12b**	**BC12c**	**BC12d**	**BC12e**	**STD**
1	*Candida albicans*	5	4	8	9	12	7	6	10	12	9	12
2	*Aspergillus niger*	6	5	7	10	13	8	8	7	13	9	13
3	*Aspergillus fumigates*	5	6	6	9	12	6	7	5	12	10	12
4	*Aspergillus parasites*	6	7	6	8	13	8	7	8	11	12	13

STD- Clotrimazole (5µg\disc)

SERIAL DILUTION METHOD

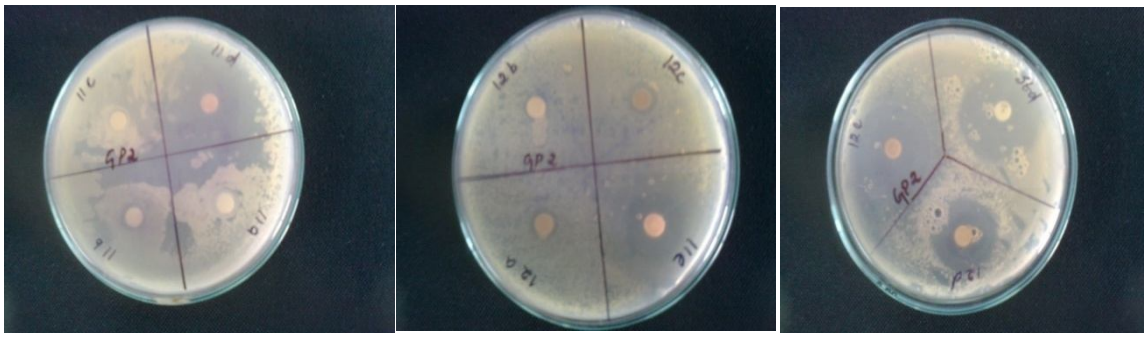
Table 9: MIC values of the synthesized compounds

S.N	Micro organism	MIC values (µg/ml)										
		BC11a	**BC11b**	**BC11c**	**BC11d**	**BC11e**	**BC12a**	**BC12b**	**BC12c**	**BC12d**	**BC12e**	**STD**
1	*Candida albicans*	100	100	25	12.5	6.25	50	50	25	12.5	25	1.9
2	*Aspergillus niger*	50	100	25	12.5	6.25	25	25	50	6.25	12.5	1.9
3	*Aspergillus fumigates*	100	50	50	25	12.5	50	25	50	12.5	12.5	2.6
4	*Aspergillu .parasites*	50	25	50	25	12.5	25	25	25	6.25	6.25	1.5

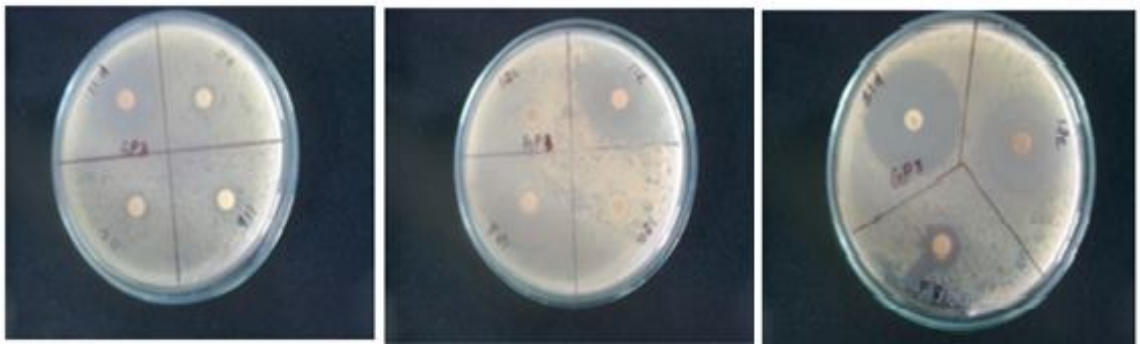
STD- Clotrimazole (100µg\ml)

ANTIBACTERIAL ACTIVITY

***Micrococcus luteus* (Gram positive)**

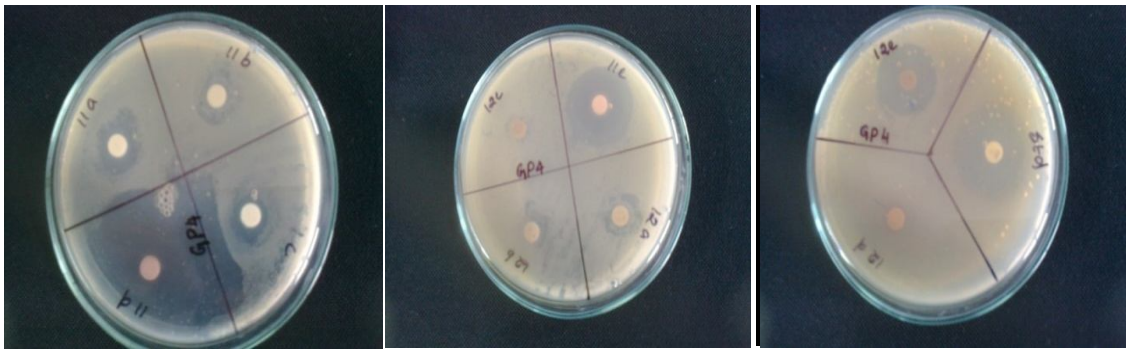


***Staphylococcus aureus* (Gram positive)**

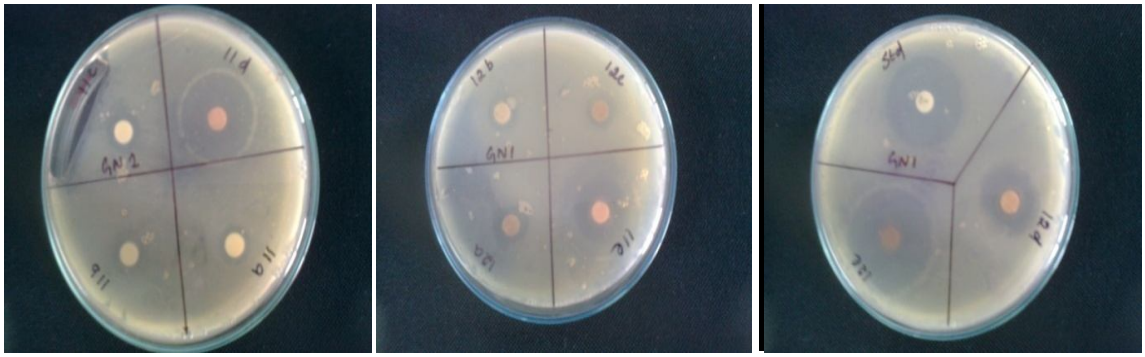


Corny bacterium diphtheria (*Gram positive*)

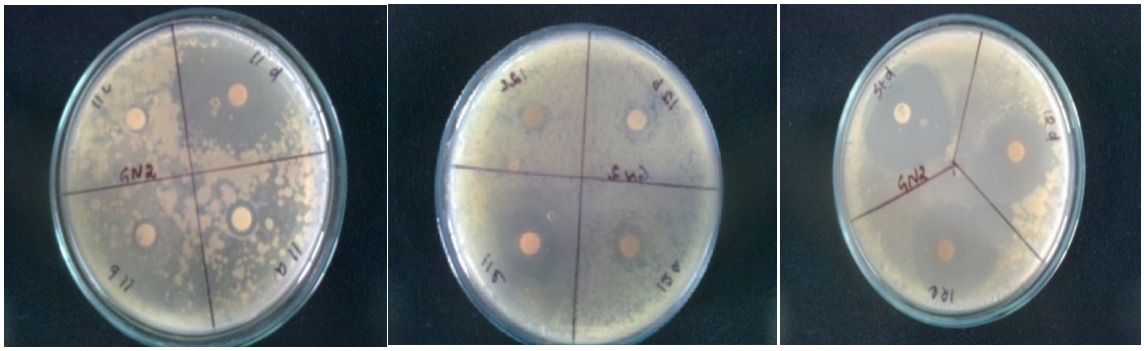
Fig: 32



***Bacillus subtilis* (Gram positive)**

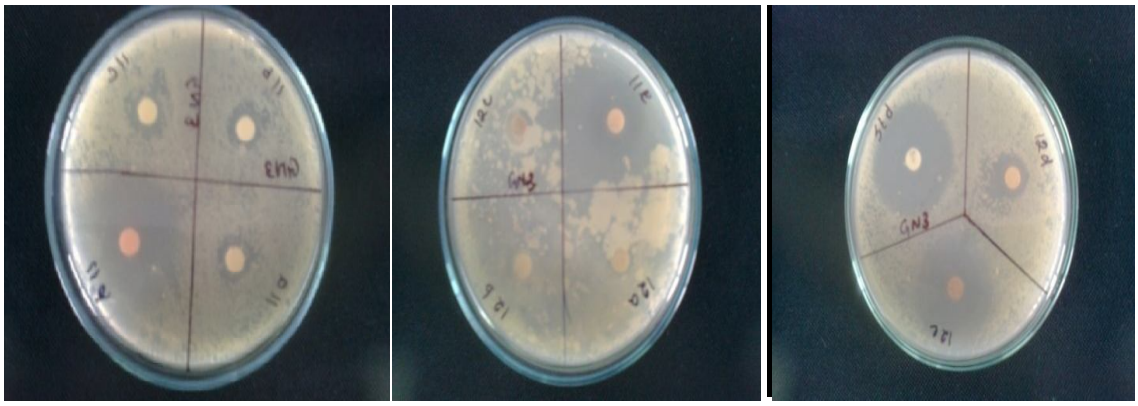


***Bacillus lintus* (Gram negative)**

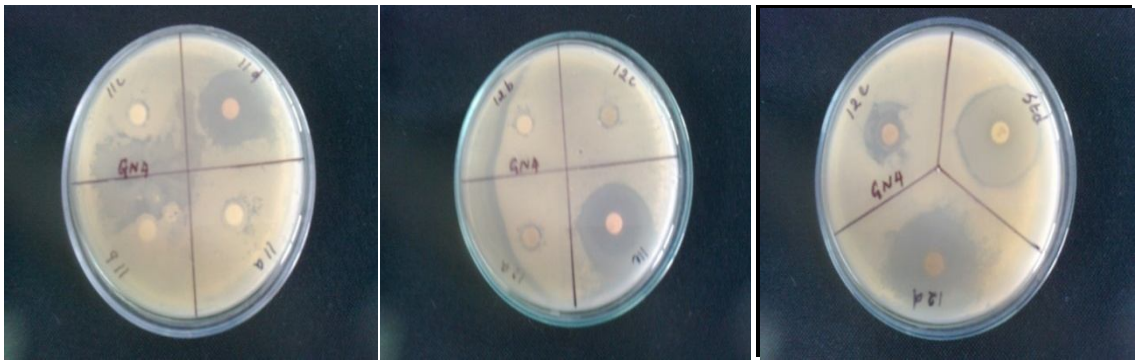


***Escherichia coli* (Gram negative)**

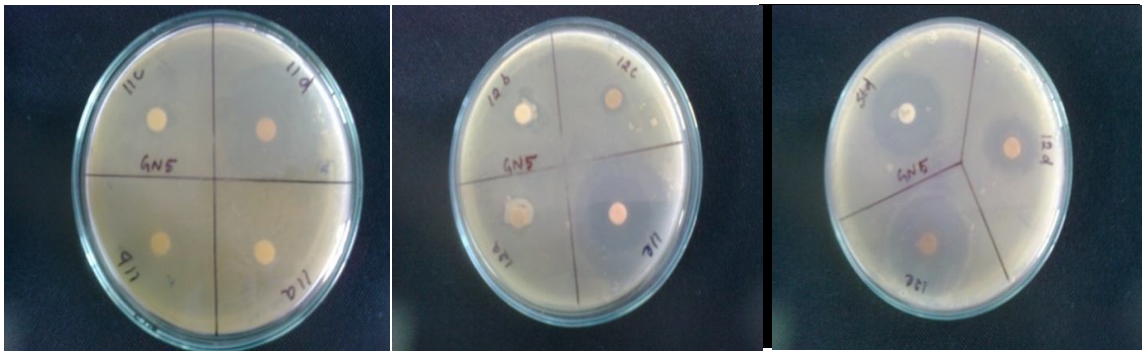
Fig: 33



***Pseudomonas aeruginosa (*Gram negative)**



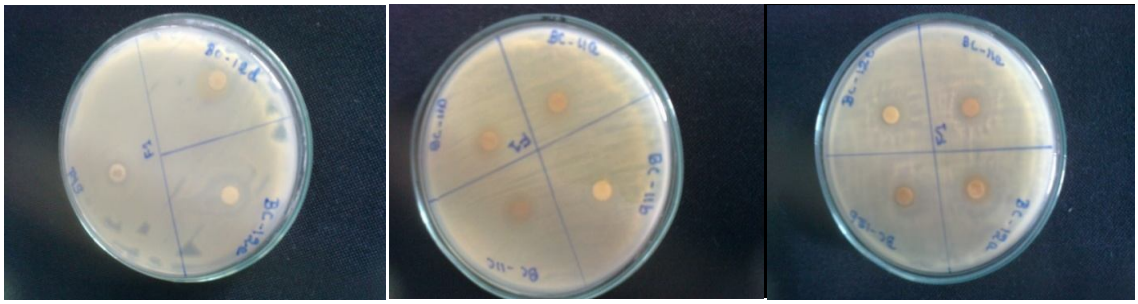
***Rhodosporium rubrum (*Gram negative)**



***Vibrio cholera (*Gram negative)**

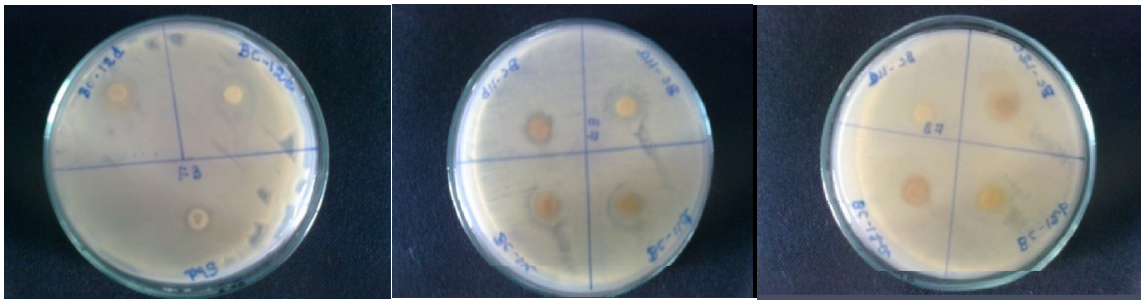
Fig: 34

ANTIFUNGAL ACTIVITY

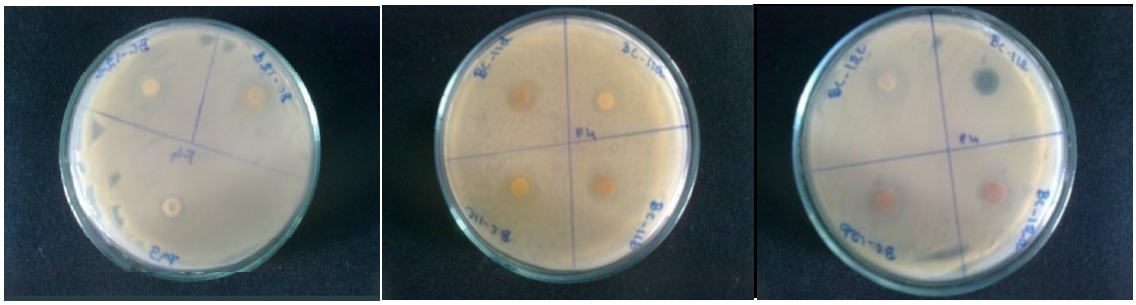


Candida albicans

Aspergillus niger



Aspergillus fumigates



Aspergillus parasiticus

Fig: 35

3.10. *IN-VITRO* ANTIOXIDANT[18]

The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay was first described by Blois in 1958 and was later modified slightly by numerous researchers. DPPH is a stable free radical that reacts with compound that can donate a hydrogen atom. This method is based on the scavenging of DPPH through the addition of a radical species or an antioxidant that change the color of DPPH solution from purple to yellow as the radical is quenched by the antioxidant.

Reagents used

Radical : DPPH

Solvent : DMSO

Standard : Ascorbic acid

Sample : compounds BC 11a-BC 11e and BC 12a-BC 12e.

Preparation of 0.3 mM DPPH solution

It was prepared by dissolving DPPH (5.91 mg) in 50 ml of ethanol. This solution was prepared freshly and kept in the dark at an ambient temperature when not used.

Preparation of sample stock solution

The sample stock solution was prepared by dissolving the compound in suitable solvent (DMSO) with a final concentration of 1mg\ml.

Preparation of standard stock solution

The standard stock solution was prepared by dissolving the Ascorbic acid in suitable solvent (ethanol) with a final concentration of 1mg\ml.

3.10.1. PROCEDURE

The effect of compound on DPPH radical was assayed by using the DPPH assay method. Sample stock solution (1mg\ml) was diluted to appropriate final concentration in DMSO. An ethanolic solution of 1ml of DPPH (0.3 mM) was added to 0.5 ml of compound and allowed to react at room temperature in a dark place for 30 minutes. After 30 minutes the absorbance values were measured at 518 ηm. All the measurements were taken as a triplicate values. From the average of the absorbance values, lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The DPPH radical scavenging capability was calculated using the following equation.

$$\textbf{\% inhibition} = (\textbf{ABS}_{\textbf{control}} - \textbf{ABS}_{\textbf{test}}) \setminus \textbf{ABS}_{\textbf{control}} \times \textbf{100}$$

Where, $ABS_{control}$ = Absorbance of ethanol + DPPH

ABS_{test} = Absorbance of DPPH + Compound\Standard

The percentage antioxidant activity (% inhibition) was extrapolated against concentration of the compound and EC_{50} was calculated by using graphical method.

3.10.2. RESULT

Antioxidant study was performed by DPPH assay method using the compounds (**BC11a-BC11e & BC12a-BC12e).**

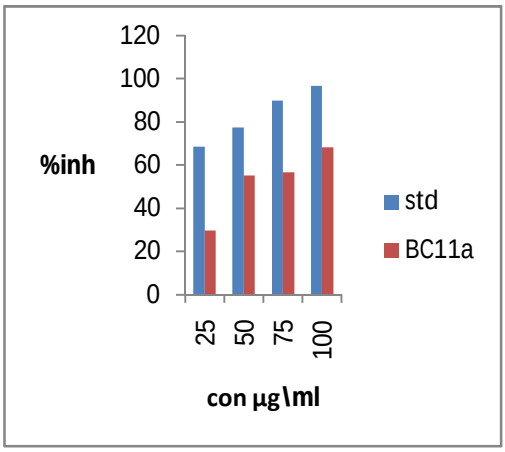


Fig: 36 % inhibition of compound BC11a

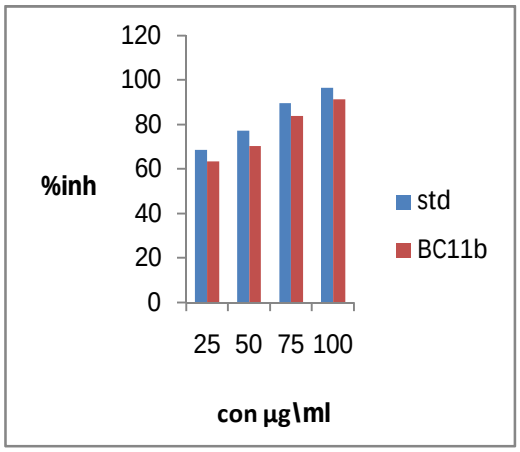


Fig: 37 % inhibition of compound BC11b

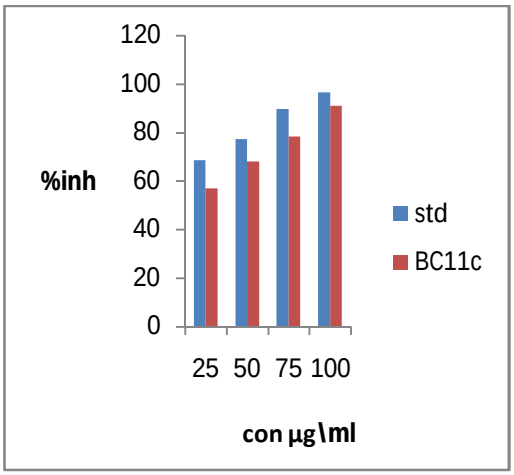


Fig: 38 % inhibition of compound BC11c

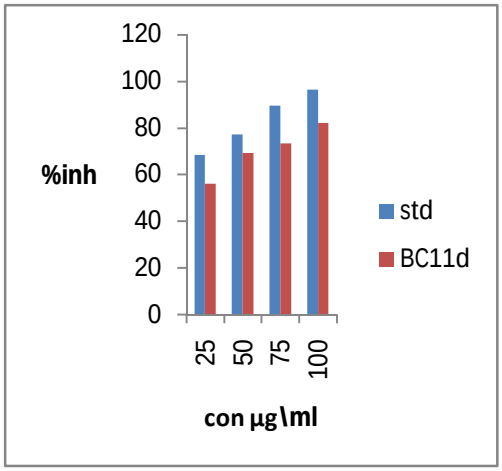


Fig: 39 % inhibition of compound BC11d

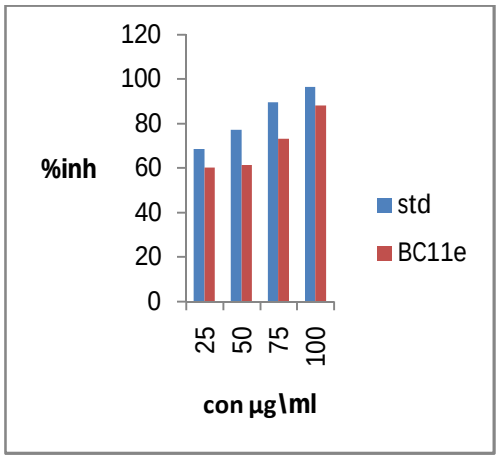


Fig: 40 % inhibition of compound BC11e

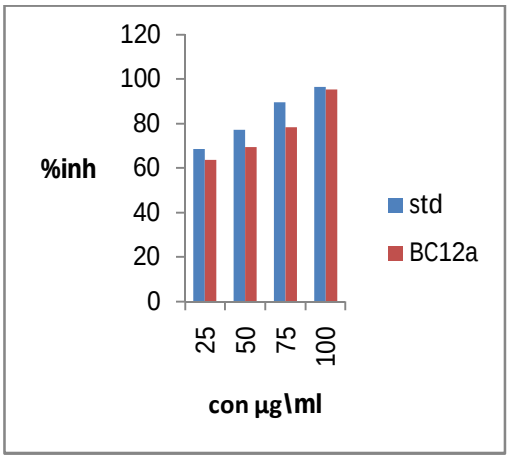


Fig: 41 % inhibition of compound BC12a

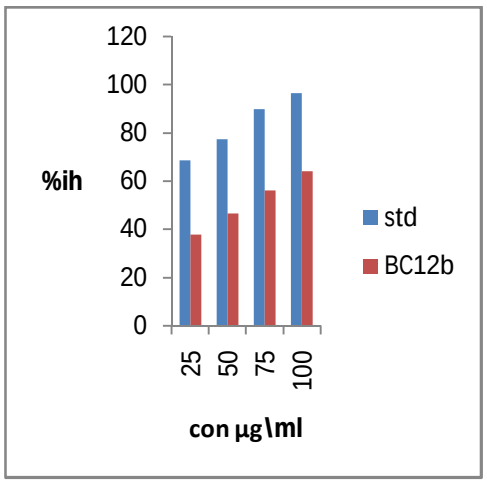


Fig: 42 % inhibition of compound BC12b

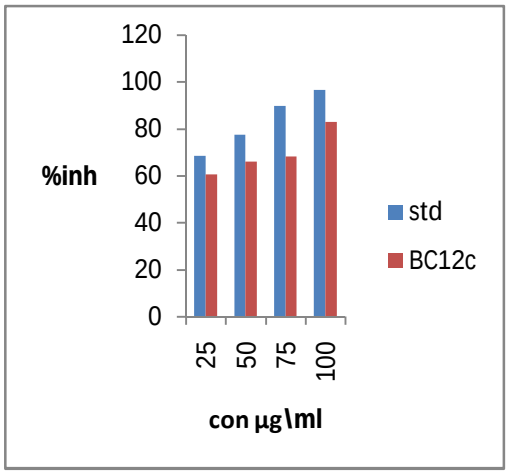


Fig: 43 % inhibition of compound BC12c

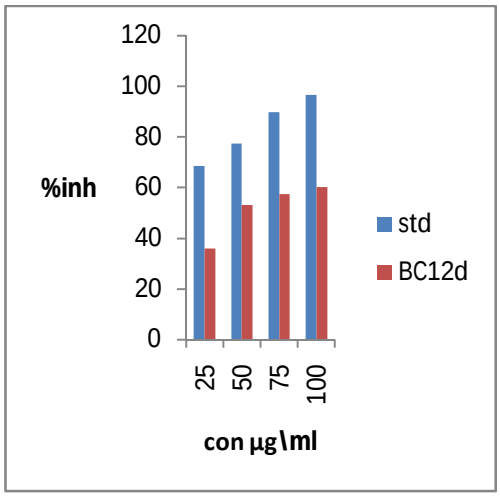


Fig: 44 % inhibition of compound BC12d

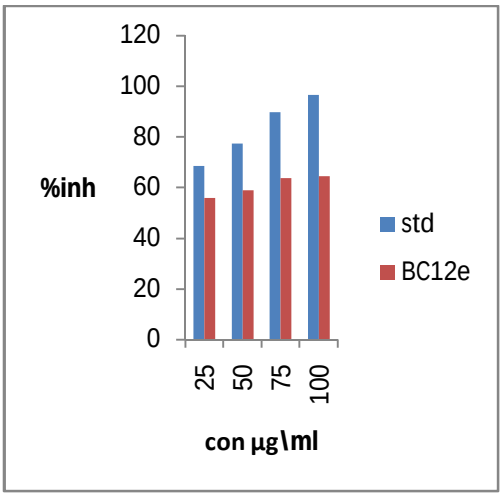


Fig: 45 % inhibition of compound BC12e

Table 10: EC_{50} value of the synthesized compounds

S.N	Compounds	% Inhibition				EC_{50} (µg)
		25µl	50 µl	75 µl	100 µl	
1	BC 11a	29.53	55.20	56.73	68.25	66
2	BC 11b	55.87	58.81	63.72	64.48	-
3	BC 11c	37.98	46.11	56.18	63.11	-
4	BC 11d	57.06	68.16	78.24	90.97	18
5	BC 11e	60.28	61.43	73.22	88.16	32
6	BC 12a	35.91	53.05	57.28	60.04	-
7	BC 12b	56.26	69.19	73.49	82.31	20
8	BC 12c	60.64	65.98	68.16	83.03	60
9	BC 12d	63.72	69.43	78.24	95.39	10
10	BC 12e	63.56	70.25	83.79	91.32	11
11	Ascorbic acid	68.48	77.35	89.71	96.46	22

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Chapter 4

Results & Discussion

RESULTS AND DISCUSSION

Chemistry and Synthesis

2-aminobenzothiazole-6-carboxylic acid (BC 1) was prepared from *p* -aminobenzoic acid based on previously reported procedures. Basic hydrolysis of BC 1 was done followed by neutralization with hydrochloric acid and anhydrous Zinc chloride to yield Zinc salt of 4-amino-3-mercaptobenzoic acid (BC 2).

The synthesis of ester derivatives of 2-{4-[*N*'-(2-carboxyethyl) carbamimidamido] phenyl}-1, 3-benzothiazole-6-carboxylic acid (BC 11a – BC 11e) are described in Series I. As shown in Series I, compound BC 2 have been coupled immediately with *p*-nitrobenzoyl chloride to yield 2-(4-nitro-phenyl) Benzothiazole-6-carboxylic acid (BC 3). Then the later was converted into acyl chloride (BC 5) followed by reduction with tin chloride to yield 2-(4-amino-phenyl)-benzothiazole-6-carbonyl chloride (BC 7), which was coupled with ammoniumthiocyanate to yield 2-[4-(carbamothioylamino) phenyl]-1, 3-benzothiazole-6-carboxylic acid (BC 9), which was further coupled with β-alanine and triethylamine in tetrahydrofuran to yield 2-{4-[*N*'-(2-carboxyethyl) carbamimidamido] phenyl}-1, 3-benzothiazole-6-carboxylic acid (BC 11). Esterification of BC 11 with alcohol derivatives with Con.sulphuric acid to yield the ester derivatives (BC 11a – BC 11e).

The synthesis of ester derivatives of 2-{4-[*N*'-(2-carboxyethyl) carbamimidamido] methyl}-1, 3-benzothiazole-6-carboxylic acid (BC 12a – BC 12e) are described in Series II. As shown in Series II, compound BC 2 have been coupled immediately with bromoacetylbromide to yield 2-(bromo methyl) Benzothiazole-6-carboxylic acid (BC 4). Then the later was converted into acyl chloride (BC 6) followed by ammonolysis with ammonia to yield 2-(4-amino-methyl)-benzothiazole-6-carbonyl chloride (BC 8), which was coupled with ammoniumthiocyanate to yield 2-[4-(carbamothioylamino) methyl]-1, 3-benzothiazole-6-carboxylic acid (BC 10), which was further coupled with β-alanine and triethylamine in tetrahydrofuran to yield 2-{4-[*N*'-(2-carboxyethyl) carbamimidamido] methyl}-1, 3-benzothiazole-6-carboxylic acid (BC 12). Esterification of BC 12 with alcohol derivatives with Con.sulphuric acid to yield the ester derivatives (BC 12a – BC 12e).

Physical properties

Melting point

Melting points were carried in open capillaries on Thomas However melting point apparatus which are uncorrected. A synthesized compounds melting point and its reactant melting points were recorded. A reactants and products melting point were differing from each others. It is clearly indicates that the formation of a new chemical entities. Melting points were given in table-1.

Solubility

Solubility properties of synthesized compounds were determined in various solvents and it was found that all compounds were completely soluble in DMSO, partially soluble in ethanol, chloroform and insoluble in water.

Percentage of yield

Percentage of yield of all synthesized compounds was calculated and values are given in the table-1.

C Log P

C Log P was calculated for all newly synthesized compounds using Chemsketch free version software and values are presented in the table-1.

Thin layer chromatography

Thin layer chromatography was performed for all newly synthesized compound as well as the parent compounds, all synthesized compounds gave a single spot whose R_f values are different from their reactants. It ultimately shows that completion of reaction and purity. The R_f values are summarized in table-2.

IR-Spectroscopy

Infra red spectroscopy was taken for all newly synthesized compounds. The characteristic peaks were observed for all the relevant groups.

The absorption peaks around 1635.07cm^{-1} & 670.14cm^{-1} indicates that the formation of C=N & C-S stretching of Benzothiazole. The C=N peak was disappeared in step II. Peaks around 1523-1550cm^{-1}& 650-700cm^{-1} were appeared for aromatic nitro compounds & aliphatic bromo compounds. This was disappeared in step 5 by the formation of amine, absorbs at 3200-3400cm^{-1}. Carboxylic acid strongly absorbs at 2500-3500cm^{-1} which was disappeared in step 4. Due to the formation of acid chloride absorbs at 1800cm^{-1}(C=O) &850.02 (C-Cl). Peaks around 2070-2085cm^{-1} was appeared for R-N=C=S stretch. Peaks around 1630-1650cm^{-1} (C=N), 2900-2800 cm^{-1}(CH_2) & 2500-3500cm^{-1}(COOH) indicates the formation of Guanidinopropionic acid in step 6. The COOH peak was vanished in step 7 by the formation ester strongly absorbs at 1725-1750cm^{-1}.

^{1}HNMR Spectroscopy

^{1}H nuclear magnetic spectra were taken for selected synthesized compounds. An aromatic proton of Benzothiazole was appeared in all the synthesized compounds around 8.39-7.14 δppm (m, 5H). Similarly phenyl & tolyl aromatic proton was observed in the region of 7.04- 6.95 δppm. Guanidine NH proton was seen at 4.34-3.6 δppm (s, 1H). A proton of methenyl group was appeared in 2.6-2.0 δppm (t, 2H).

Mass Spectroscopy

The mass spectral analysis of the selected synthesized compound BC 11d & BC 12e was performed and their mass spectrum of the compound was in agreement with its molecular weight. Molecular ion, [M+2] and base peaks are given in table-4.

The characteristic [M+2] Cl isotope peak was also appeared in the spectrum.

Biological activity

In-vitro Aldose reductase inhibitory activity

The synthesized compounds were screened for their inhibitory activity against Aldose reductase. Compounds BC 12d > BC 12e > BC 11d > BC 11e have showed inhibition 80%, 68%, 59% & 53% respectively. Among these four, compound BC 12d was the most promising candidate and other compounds showed poor activity. The results are summarized in table-5.

The results indicated that alkyl substituted Benzothiazole compound (BC 12d & BC 12e) showed good inhibitory activity than aryl substituted Benzothiazole compound (BC 11d & BC 11e). On the other hand aromatic ester derivatives of Benzothiazole (BC 12d, BC 12e, BC 11d & BC 11e) improves inhibition in comparison to aliphatic ester derivatives (BC 11a, BC 11b, BC 11c, BC 12a, BC 12b & BC 12c).

Docking studies with Aldose reductase

In order to gain an insight into the binding mode of active compound BC 12d has been docked into the ligand binding pocket of Aldose reductase (PDB entry 2BGQ). Fig1 shows the corresponding interaction with ligand and protein. Results indicate hydrogen bond interactions (2.25 Å) between the carbonyl group of ester in compound and the catalytic amino acid residues Arg 265 and Ser 261.

In-vitro Antioxidant activity

Synthesized compounds were evaluated for antioxidant activity by DPPH method. Results are depicted in table-10.

In both the series compounds BC 11d, BC 11e, BC 12d & BC 12e have identified promising antioxidant activity; it could be presence of phenyl & *o*- tolyl substituent at 3rd position of Guanidinopropionate. The antioxidant results depicted that the electron donating methylene linker derivatives have better activities than its counterpart.

In-vitro Antimicrobial

The synthesized compounds were screened for *in vitro* antibacterial and antifungal activity by Disc Diffusion and Serial Dilution technique using ciprofloxacin and clotrimazole drugs as reference standard. Results were presented in (table 7-9).

Antibacterial activity

Compound BC 11d, BC 11e, BC 12d & BC 12e MIC (12.5 µg\ml) gave the moderate activity against *Micrococcus lutes.*

Compounds BC 11d, BC 11e, BC 12d & BC 12e (MIC 12.5 µg\ml) showed moderate activity against *Bacillus linctus.*

Compounds BC 11c, BC 11d, BC 11e, BC 12d & BC 12e (MIC 12.5 µg\ml) posses moderate activity against *Staphylococcus aureus.*

Compounds BC 11b, BC 11c, BC 11d, BC 11e, BC 12d & BC 12e (MIC 12.5 µg\ml) thesaurus moderate activity against *Bacillus substilis.*

Compounds BC 11c, BC 11d, BC 11e, BC 12d & BC 12e (MIC 12.5µg\ml) displayed moderate activity against *Salmonella paratyphi.*

Compounds BC 11d, BC 11e, BC 12d & BC 12e (MIC 12.5µg\ml) showed moderate activity against *Pseudomonas aeuroginosa.*

Compounds BC 11d, BC 11e, BC 12d & BC 12e (MIC 12.5µg\ml) displayed moderate activity against *Vibrio cholera.*

Compounds BC 12d & BC 12e (MIC 6.25µg\ml) expressed moderate activity against *E.coli.*

When compared to all the compounds, BC 11d, BC 11e BC 12d & BC 12e possess antibacterial activity against all the tested bacteria but less potent than the Standard. This activity might be due to the presence of phenyl & *o*-tolyl substituent at 3rd position of guanidinopropionate.

Antifungal activity

Compound BC 11e (MIC 6.25µg\ml) revealed moderate activity than the other compounds against *Candida albicans*.

Compounds BC 11e & BC 12d (MIC 6.25µg\ml) exhibited moderate activity than the other compounds against *Aspergillus niger*.

Compounds BC 11e, BC 12d & BC 12e (MIC 12.5µg\ml) have been showed moderate activity than the other compounds against *Aspergillus fumigates*.

Compound BC 12d & BC 12e (MIC 6.25µg\ml) displayed moderate activity than the other compounds against *Aspergillus parasites.*

When compared to all the compounds, BC 11e & BC 12d compound possess antifungal activity against all the tested fungi but not superior than the standard. This activity might be due to the presence of o phenyl & *o*-tolyl substituent at 3rd position of guanidinopropionate.

Chapter 5

Conclusion

CONCLUSION

A new series of Benzothiazole fused Guanidinopropionic ester derivatives have been synthesized and evaluated for their potency as Aldose reductase inhibitors. Of those, compound BC 12d depicted as a potent compound with promising activity. Additionally, it exhibited a high antioxidant potential to inhibit the formation of advanced glycation end products. Docking results suggested that compound BC 12d form hydrogen-bond interactions (2.25 Å) with the catalytic amino acid residues Arg 265 and Ser 261 of Aldose reductase.

Further studies on its possible mechanism and *in-vivo* trials in experimental animals to broaden their pharmacological assessment.

ABSTRACT

A novel series of Benzothiazole fused Guanidinopropionic ester derivatives have been synthesized and characterized by FTIR, ^{1}HNMR and mass spectral analysis. All the compounds were evaluated for their Aldose reductase (enzyme inhibition assay) and DPPH free radical (Scavenging assay) further more evaluated for antimicrobial activities (Disc diffusion and MIC method) against some pathogenic bacteria and fungi. Results revealed that, compound BC 12d exhibited promising AR inhibitory (80% inhibition, 10µm), free radical scavenging (95% inhibition) and antimicrobial activities (MIC 12.5-6.25µg\ml) at varied concentrations. The most active compound BC 12d has been docked into the crystal structure of Aldose reductase. Docking results indicate hydrogen bond interactions (2.25 Å) between the carbonyl group of ester in compound and the catalytic amino acid residues Arg 265 and Ser 261.